Pathophysiological basis for the prophylaxis of preeclampsia through early supplementation with antioxidant vitamins

Ramón Rodrigo[a,*], Mauro Parra[b], Cleofina Bosco[c], Virginia Fernández[a], Pilar Barja[d], José Guajardo[a], Rodrigo Messina[a]

[a]*Molecular and Clinical Pharmacology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Independencia 1027, Casilla 70058, Santiago 7, Chile*

[b]*Fetal Medicine Unit, Department of Obstetrics and Gynaecology, University of Chile Clinical Hospital, Santos Dumontt 999, Independencia, Santiago, Chile*

[c]*Anatomy and Biology of Development Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Independencia 1027, Casilla 70079, Santiago 7, Chile*

[d]*Pathophysiology Program, Institute of Biomedical Sciences, Faculty of Medicine, University of Chile, Independencia 1027, Casilla 70058, Santiago, Chile*

Abstract

Preeclampsia (PE) is a multisystem disorder that remains a major cause of maternal and foetal morbidity and death. To date, no treatment has been found that prevents the development of the disease. Endothelial dysfunction is considered to underlie its clinical manifestations, such as maternal hypertension, proteinuria, and edema; however, the precise biochemical pathways involved remain unclear. A current hypothesis invokes the occurrence of oxidative stress as pathogenically important, as suggested by the fact that in PE, the placental and circulating levels of lipid peroxidation products (F2-isoprostanes and malondialdehyde [MDA]) are increased and endothelial cells are activated. A potential mechanism for endothelial dysfunction may occur via nuclear transcription factor kappa B (NF-κB) activation by oxidative stress. Alternatively, the idea that the antiangiogenic placental soluble fms-like tyrosine kinase 1 factor (sFlt1) is involved in the pathogenesis of this disease is just emerging; however, other pathophysiological events seem to precede its increased production. This review is focused on evidence providing a pathophysiological basis for the beneficial effect of early antioxidant therapy in the prevention of PE, mainly supported by the biological effects of vitamins C and E.

Keywords: Preeclampsia; Oxidative stress; Endothelial dysfunction; Vitamin E; Vitamin C

Abbreviations: ADMA, asymmetric dimethyl arginine; ARE, antioxidant responsive element; BH_4, tetrahydrobiopterin; eNOS, endothelial nitric oxide synthase; ET, endothelin; Hcy, homocysteine; HDL, high-density lipoprotein; HUVEC, human umbilical vascular endothelial cells; ICAM-1, Intercellular adhesion molecule 1; IL-6(8), interleukin-6(8); iNOS, inducible nitric oxide synthase; LDL, low-density lipoprotein; L-NAME, *N*(omega)-nitro-L-arginine methyl ester; MDA, malondialdehyde; NF-κB, nuclear factor-kappa B; NO, nitric oxide; oxLDL, oxidised low-density lipoprotein; PE, preeclampsia; PlGF, placental growth factor; ROS, reactive oxygen species; sFlt1, soluble fms-like tyrosine kinase 1; SOD, superoxide dismutase; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor.

Contents

* Corresponding author. Laboratorio de Fisiopatología Renal, Programa de Farmacología Molecular y Clínica, Facultad de Medicina, Universidad de Chile, Independencia 1027, Casilla 70058, Santiago 7, Chile. Tel.: +56 2 6786126; fax: +56 2 7372783.

E-mail address: rrodrigo@med.uchile.cl (R. Rodrigo).

1. Introduction

Preeclampsia (PE), a multisystem syndrome that affects between 0.4% and 2.8% of all pregnancies in the developed countries (Villar et al., 2003), is associated with substantial maternal and neonatal morbidity and mortality (Roberts & Cooper, 2001; Davison et al., 2004). There is no satisfactory treatment to prevent the development of the disease, except steps to avoid or reduce complications such as hypertension and eclamptic seizures (Afifi & Churchill, 2003), and early delivery is the only successful treatment. The primary event of PE remains largely unknown, but endothelial dysfunction plays a major role in the underlying pathophysiological mechanism of the disease. Most of the clinical attributes of PE, such as hypertension, proteinuria, and edema, are derived from pathological changes within the maternal vascular endothelium or involve endothelial dysfunction (Roberts, 1998). The endothelium participates in the modulation of vascular tone, control of primary hemostasis, host defence and inflammation, transport of nutrients and other solutes, and activation/inactivation of various vasoactive hormones. Endothelial dysfunction is characterised by a shift of the actions of the endothelium toward reduced vasodilation, a proinflammatory state, and prothrombic properties (Endemann & Schiffrin, 2004). Factors likely playing a role in the impairment of normal endothelial cell function in PE include placental ischemia, lipoprotein-induced toxicity, increased reactive oxygen species (ROS), antiangiogenic effects, and immune maladaptation, which result in the synthesis and release of proinflammatory cytokines. Although the nature of this disease seems to be multifactorial, accumulated evidence indicates that oxidative stress may represent a point of convergence for several contributing factors potentially leading to endothelial cell dysfunction and eventually to the clinical manifestations of PE. Pregnancy per se leads to oxidative stress (Wisdom et al., 1991), due to an increased mitochondrial activity, reduced antioxidant scavenging potential, and occurrence of ischemia–reperfusion events, in the placenta. In agreement, heightened level of oxidative stress status was encountered (Myatt & Cui, 2004) and confirmed in most studies. However, this view has been recently questioned because of the finding of less profoundly increased levels of oxidative stress status in preeclamptic women, as assessed by several biochemical parameters in women with PE compared with normotensive pregnant women (Llurba et al., 2004). Supplementation with vitamins C and E, 2 antioxidant agents, in women at increased risk of PE, has been associated with normalisation of plasma markers of vascular endothelial activation, placental insufficiency, and the occurrence of the disease (Chappell et al., 1999). Accordingly, it was suggested that this treatment might be beneficial in the

prevention of PE, although the rationale of this prophylactic approach needs to be investigated further. The aim of the present review is to present the pathophysiological and molecular evidence supporting a role of antioxidant vitamins C and E as potential therapeutic agents in the prophylaxis of PE.

2. Oxidative stress in normal pregnancy

Pregnancy itself is a condition of increased susceptibility to oxidative stress, leading to potential tissue damage (Sies, 1991). Several organs in pregnant women show increased basal oxygen consumption and changes in substrate energy use. The production of ROS within the foetal–placental unit has special relevance due to its highly vascularised nature and since the tissue is rich in mitochondria, there is also an abundance of macrophages. At pregnancy term, the human placenta is hemomonochorial, meaning that only 1 chorionic trophoblast cell layer exists between maternal and foetal blood, which favours the exchange of gases, nutrients, and metabolic products. In turn, oxygen delivery is favoured by the lower partial pressure of oxygen, which raises lactic acid production and lowers pH, within the placental cells and foetal circulation. In addition, as the hypoxic placenta matures, vascularisation develops to create an oxygen-rich environment. Moreover, the increasing mitochondrial mass favours the production of ROS (Liochev & Fridovich, 1997). Nitric oxide (NO) is also produced by the placenta (Dotsch et al., 2001), giving rise to another local source of free radicals, likely contributing to endothelial dysfunction, as will be further discussed below. Finally, because the placenta is also rich in macrophages (Hofbauer cells), the local production of free radicals, including reactive chlorine species, also might contribute to the development of oxidative stress (Myatt & Cui, 2004). This view is based on the ability of Hofbauer cells to express both inducible nitric oxide synthase (iNOS) activity (Myatt et al., 1997) and NADPH oxidase activity (Matsubara et al., 2001), although studies on xanthine oxidase are still lacking. On the other hand, the defence mechanisms against ROS damage are also enhanced in pregnancy. Thus, a progressive increase in free radical scavengers, such as glutathione and bilirubin, as well as in the activity of antioxidant enzymes in placenta has been reported (Watson et al., 1997, 1998; Qanungo & Mukherjea, 2000). In addition, the activities of glutathione peroxidase in erythrocytes and platelets and of extracellular superoxide dismutase (SOD) have also been found to increase progressively throughout gestation up to the third trimester (Uotila et al., 1991; Tamura et al., 2001), which suggests that there is an increased presence of superoxide anions. In agreement with these observations, the induction of antioxidant enzymes after exposure to oxidative stress is well documented (Talalay et al., 2003; Bae et al., 2004). Genes encoding these enzymes are often coordinately regulated through antioxidant responsive element (ARE) in their gene regulatory regions (Lee & Johnson, 2004). Furthermore, it has been reported that transcription factor NF-E2-related (Nrf2) binds to these ARE sites, leading to the up-regulation of downstream genes that regulate a wide array of ARE-driven genes in various cell types. To compensate for these effects, lipid peroxidation becomes more attenuated, with the progress of gestation, protecting the foetus against ROS toxicity (Qanungo & Mukherjea, 2000).

Adaptive mechanisms enhancing the maternal antioxidant defence system that counteract the effect of free radicals through enzymatic induction, as well as through nonenzymatic free radical protectors and scavengers, could prevent the occurrence of oxidative stress. However, pregnancy is a state where this adaptation and equilibrium could be easily disrupted. Thus, oxidative damage has been implicated in preterm premature rupture of membranes (Woods et al., 2001) and is associated with intrauterine growth restriction (Takagi et al., 2004). It seems likely that vitamins C and E could exert a protective effect by the reinforcement of antioxidant defences. An in vitro study reported evidence that vitamins C and E protect the strength and integrity of the chorioamnion from ROS-induced damage (Plessinger et al., 2000).

The antioxidant nutrient balance in pregnant women could also play an important role in foetal growth. Although little is known about the maternal and foetal pharmacodynamics of vitamins C and E, recent studies in full-term babies reported that birth weight and length were highest when the levels of both vitamins C and E were high (Lee et al., 2004). The authors also found that maternal serum levels of vitamin C, during the second trimester of pregnancy, were positively correlated with birth weight and length of these babies. However, when the gestational age was further controlled in the regression model, the association between vitamin C level and birth weight did not reach statistical significance. Therefore, further research may be needed to verify the role of vitamins C and E during foetal growth. Moreover, the effect of these vitamins is exerted in reinforcing the embryonic antioxidant defence mechanism, possibly during the first trimester of gestation (Zaken et al., 2001), mainly since the placenta has a limited antioxidant enzyme capacity during this period (Jauniaux et al., 2004).

Deficient invasion of the endometrium by extravillous cytotrophoblast cells during the first trimester of pregnancy is the most widely recognised predisposing factor for PE (Hubel, 1999). The normal process consists of the physiological conversion of highly tortuous and thick-walled vessels to flaccid sinusoidal conduits of low resistance, but in PE, this is associated with a partial failure of placental trophoblast invasion. Consequently, spiral arteries display an abnormally high vascular resistance with reduced uteroplacental perfusion as confirmed by Doppler flow velocimetry studies (Aquilina & Harrington, 1996).

The generation of lipid peroxides in PE is thought to be initiated in the placenta through an exaggerated production

of superoxide anion that reacts rapidly with NO to form peroxynitrite. Accordingly, significantly elevated levels of lipid peroxidation products, such as malondialdehyde (MDA) and F2-isoprostanes (8-Iso-$PGF_{2\alpha}$), have been found in preeclamptic placental tissue (Staff et al., 1999; Madazli et al., 2002), as well as in the plasma and urine of preeclamptic women (Kumar & Das, 2000; McKinney et al., 2000; Barden et al., 2001). In addition, the highest levels of free 8-Iso-$PGF_{2\alpha}$ were found in severe cases of PE. This finding has special relevance because 8-Iso-$PGF_{2\alpha}$, besides being a potent vasoconstrictor, is the result of peroxidation of arachidonic acid, which is the precursor of the substantial vasodilator prostacyclin (PGI_2). Lipid peroxides, oxidative stress, and ROS have been shown to activate nuclear transcription factor kappa B (NF-κB) in endothelial vascular cells (Takacs et al., 2001), a mediator responsible for the activation of proinflammatory cytokines and chemokines (Kauma et al., 2002). The addition of 50 mM vitamin E, a dose comparable with serum levels that result from taking 400 IU of vitamin E orally, inhibited NF-κB activation in human umbilical vascular endothelial cells (HUVEC) (Takacs et al., 2001). These studies support the hypothesis that the beneficial effects of antioxidant therapy in the prevention of PE occur through the inhibition of the lipid peroxide activation of vascular endothelial cells.

3. Oxidative stress in preeclampsia

Although the cause of PE remains largely unknown, the occurrence of oxidative stress is a feature of the maternal syndrome. The main source of ROS initiating the pathophysiological events appears to be the placenta (Serdar et al., 2002), but maternal leukocytes and maternal endothelium are also likely contributors. In addition, it has been proposed that oxidative stress is a component of PE that could provide the linkage between decreased placental perfusion and the maternal syndrome (Roberts & Hubel, 1999; Moretti et al., 2004).

3.1. Pathophysiology

Reduced placental perfusion, due to abnormal vascular development of the blood vessels in preeclamptic placenta, is secondary to deficient trophoblast invasion (Burton & Jauniaux, 2004) that may also alter the implantation (Merviel et al., 2004). Thus, spiral arteries display an abnormally high vascular resistance with reduced uteroplacental perfusion as confirmed by Doppler flow velocimetry studies (Aquilina & Harrington, 1996). The inadequacy of placental perfusion is likely to result in oxidative stress through an intermittent placental perfusion due to changes in the vascular vasomotor activity by maternal humoral and neural influences (Burton & Jauniaux, 2004), giving rise to hypoxia–reoxygenation cycles. As will be further discussed below, hypoxia is a potent stimulus of ROS generation. Also, as a result, the activation of apoptotic pathways could lead to increased deportation of syncytiotrophoblast microvesicles into the maternal circulation. These particles have been directly linked to activation of maternal neutrophils, although maternal neutrophils may also be locally activated during the passage of maternal blood through the placenta (Raijmakers et al., 2004a). Thus, isolated neutrophils from women with PE synthesise more superoxide than those of normotensive pregnant women. In turn, activated neutrophils may contribute to the activation of the vascular endothelium. Maternal blood leukocytes in preeclamptic patients are activated and support the view that oxidative stress is a contributing factor in the pathophysiology of PE (Holthe et al., 2004).

In addition, the problems caused by perfusion could be the result of activation of a coagulation cascade, especially in platelets, with microthrombi formation. It is known that the various maternal constitutional factors, together with decreased placental perfusion, result in the generation of oxidative stress. The toxaemia theory, proposing that the compromised placenta produces substances that lead to the maternal syndrome of PE, has gained validity in the light of more recent data showing an increased concentration of the products of oxidative stress and is able to account for the pathophysiological sequence of events.

3.2. Prooxidant enzymes

The most common ROS is the superoxide anion, generated in living cells by NADPH oxidase, xanthine oxidase, flavin enzymes, and enzymes in the mitochondrial electron transport chain. Xanthine and NADPH oxidases have been identified as major vascular superoxide-forming enzyme systems, but the contribution of xanthine oxidase is generally minor (Poston & Raijmakers, 2004). Ischemia–reperfusion is a potent stimulus to the conversion of xanthine dehydrogenase to xanthine oxidase, which is abundantly expressed in cytotrophoblast, syncytiotrophoblast, and villous stromal cells (Hassoun et al., 1994; Many et al., 2000). Therefore, xanthine oxidase is likely to play a key role in tissue damage in the human placenta, evidenced by an increase in nitrotyrosine staining in trophoblast and activation of apoptotic pathways, but these changes are abolished by the addition of a free radical scavenger (Hung et al., 2002). In turn, NADPH oxidase activity, constitutively observed in the trophoblast of the human placenta, is massively stimulated in PE (Matsubara & Sato, 2001). This enzyme consists mainly of 5 subunits ($p22^{phox}$, $p47^{phox}$, $p67^{phox}$, Rac, and $gp91^{phox}$), which, under appropriate stimulation, assemble at the cell membrane. Placentas from preeclamptic patients show increased expression of NADPH oxidase components p22, p47, and p67 (Dechend et al., 2003). Other forms of NADPH oxidase are also implicated in the pathophysiology of PE, such as those present in phagocytes (neutrophilic and eosinophilic granulocytes, monocytes, and macrophages)

and vascular cells. Isolated neutrophils from women with PE synthesise more superoxide than those from normotensive pregnant women (Lee et al., 2003a), and this is mediated by NADPH oxidase (Lee et al., 2003b). Vascular NADPH oxidase plays a major role in the development of hypertension (Touyz & Schiffrin, 2004) and is a target for a down-regulation exerted by antioxidants, such as vitamins C and E. In addition, angiotensin receptor agonist antibodies, which have been isolated from the blood of women with PE (Wallukat et al., 1999), have been suggested to be responsible for the stimulation of vascular NADPH oxidase (Poston & Raijmakers, 2004). Recently, the development of hypertension in spontaneously hypertensive rats was prevented by treatment with antioxidants (Zhan et al., 2004). Accordingly, it has been demonstrated that vitamins C and E can exert a down-regulation on NADPH oxidase activity (Ülker et al., 2003) and thus could contribute to attenuate the elevation of blood pressure accompanying the syndrome of PE. Finally, it was suggested that NADPH oxidase could act as an oxygen sensor, regulating differentiation from cytotrophoblast to syncitiotrophoblast when oxygen tension increases (Manes, 2001). Studies in vitro revealed that the association of α-tocopherol (25 μM) and ascorbate (50 μM) prevents the release of lipid peroxides from placental mitochondria and therefore could be protective against the development of PE (Milczarek et al., 2000).

3.3. Placental ischemia–reperfusion and reactive oxygen species

Although biomarkers of oxidative stress have been detected in the blood of women with PE for over 40 years (Roberts & Cooper, 2001), the mechanism whereby the biological effects can mediate the generation of the syndrome has only recently attracted interest. Despite the possibility that the generation of the placental oxidative stress may be related to hypoxia derived from a reduced uteroplacental perfusion, hypoxia alone is not sufficient to account for all the morphological findings (Burton et al., 1999). In fact, the lack of placental perfusion continuity may be a more important factor because once the maternal blood flow is reestablished, an ischemia–reperfusion cycle occurs (Hung et al., 2001). As a consequence, an increased capacity of placental as well as of other cells to generate ROS has been found in PE (Many et al., 2000; Serdar et al., 2002). If the generation of ROS exceeds the capacity of the antioxidant defences, then oxidative stress will cause placental damage, which could account for the increased rates of infarction and syncytial necrosis. Therefore, because the placenta seems to be the primary target of ROS, it should be expected that the reinforcement of the antioxidant defences (through vitamins C and E supplementation) be accompanied by an amelioration of the deleterious effects derived from the placental damage.

3.4. Biomarkers of oxidative stress

The generation of lipid peroxides in PE is thought to be initiated in the placenta through an exaggerated production of superoxide anion via prooxidant enzymes. In addition, increased plasma levels of products of lipid peroxidation and protein carbonylation have been encountered, likely due to the contribution of placenta, activated leukocytes, and endothelial cells. The main biomarkers of oxidative stress will be analysed below.

3.4.1. Peroxynitrite

The increased generation of superoxide anion by the placenta, activated leukocytes, or endothelial cells is accompanied by an up-regulation of iNOS, an isoform mainly located in macrophages and neutrophils, leading to increased formation of the potent peroxidant peroxynitrite. When superoxide and NO concentrations are increased, NO effectively outcompetes SOD for superoxide, resulting in peroxynitrite anion formation. Indeed, the rate constant for the formation of peroxynitrite is 3 times faster than that of the interaction of superoxide with SOD (Huie & Padmaja, 1993). Peroxynitrite in high concentrations is cytotoxic and may cause oxidative damage to proteins, lipids, and DNA (Beckman & Koppenol, 1996). Furthermore, in the foetal vasculature and villous stroma of preeclamptic placentas, there is an increased expression of nitrotyrosine residues, formed from the interaction of peroxynitrite with tyrosine moieties (Myatt et al., 1996).

3.4.2. Malondialdehyde

The reaction of peroxynitrite with lipids leads to peroxidation and MDA and conjugated diene formation (Var et al., 2003a). In women with PE, MDA levels are reported to be elevated in maternal plasma and placental tissue (Madazli et al., 2002; Takacs et al., 2003; Aydin et al., 2004), as well as in erythrocytes. In fact, it was found that the severity of the disease did indeed correlate with both the MDA concentration in the serum (Serdar et al., 2002) and that in erythrocytes (Madazli et al., 1999). Accordingly, it was shown that the greater the level of lipid peroxidation, the greater the severity of PE (Panburana et al., 2000). Similarly, there are studies indicating a positive correlation between the severity of PE and the degree of oxidative stress, which corroborate data suggesting ROS to be involved in the pathophysiology of PE (Wiktor et al., 2004). Furthermore, an erythrocyte level of 35.98 nmol MDA/g haemoglobin or greater was demonstrated to be a value that correlated with the development of PE in the third trimester (Basbug et al., 2003). In addition, the determination of MDA in the cord was reported to be useful in recognising neonates who are at risk of asphyxia (Zeteroğlu et al., 2004). It is noteworthy also that MDA products may behave as toxic bifunctional electrophiles, due to reactivity with proteins, phospholipids, and DNA, generating stable products at the end of a series of reactions to form propane

adducts (Blair, 2001). Consequently, a change in the properties of the molecule, for example, in its charge profile, could result in modified cell–matrix interactions (Slatter et al., 2000). Lipid peroxides, normally present in lipoproteins or membranes, are known to further induce lipid peroxidation, inhibit the mitochondrial electron transport system, and to oxidise sulfhydryl groups on proteins, hence altering its function or otherwise disrupting signal transduction pathways (Ischiropoulos et al., 1992). Although the thiobarbituric acid reaction has been the most widely used method for the measurement of MDA in a wide array of samples, including the majority of data here reported, it has also been the most criticised one because of the nonspecificity and overestimation of true MDA (Liu et al., 1997). In addition, MDA is also a cyclooxygenase product. Nevertheless, despite the negative features of this method, if interpreted cautiously and correlated with data from other indices, it could offer a window to the complex process of lipid peroxidation.

3.4.3. F2-isoprostanes

The nonenzymatic peroxidation of arachidonic acid results in the formation of F2-isoprostanes, which are also products of lipid peroxidation. The levels of these compounds can serve as an index of lipid peroxidation in several diseases, and they have been reported to be significantly increased in women with PE (Hubel et al., 1996; Barden et al., 2001). The production and secretion rates of F2-isoprostanes for placentas obtained from women with PE were significantly higher than that of controls (Walsh et al., 2000), providing convincing evidence that oxidative stress and lipid peroxidation are abnormally increased in preeclamptic placenta. Although F2-isoprostanes may be generated from cyclooxygenase-2 catalysed reactions in mesangial cells and platelets (Klein et al., 1997), to our knowledge, there are no studies revealing their enzymatic formation in the placenta.

3.4.4. Carbonyls

The direct damage of proteins during oxidative stress can give rise to the formation of protein carbonyls, which may serve as biomarkers for general oxidative stress, in addition to data provided by lipid peroxidation. Higher levels of protein carbonyls in both the placenta and decidua were found in women with mild to severe PE alone (Serdar et al., 2002) and also with concurrent hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome, a rare but potentially serious complication of pregnancy (Zusterzeel et al., 2001).

Difficulties for the assessment of oxidative stress could occur due to confounders, hazard, and artifactual factors affecting the true estimation of the parameters. From the above mentioned biomarkers, likely the occurrence of oxidative stress in PE could be better reflected in the plasma levels of free F2-isoprostanes. F2-isoprostanes are a unique series of prostaglandin-like compounds formed in vivo from the free radical-catalysed peroxidation of arachidonic acid independent of the cyclooxygenase enzyme (Morrow, 2000). Because of their mechanism of formation, specific structural features that distinguish them from other free radical-generated products and chemical stability, they can provide a reliable index of lipid peroxidation in vivo in a variety of clinical settings associated with oxidative stress (Cracowski et al., 2001). In addition, this determination provides a noninvasive method for dose selection to monitor the response to antioxidant treatment (Pratico et al., 2001). Previously, F2-isoprostanes measurement has been successfully applied for the assessment of oxidative stress in clinical studies of PE (Barden et al., 1996; McKinney et al., 2000; Barden et al., 2001; Chappell et al., 2002b).

4. Role of antioxidants in preeclampsia

4.1. Antioxidant enzymes

In normal pregnancy, by 10 to 12 weeks of gestation, the onset of maternal blood flow in the placenta results in a local increase in oxygen tension and parallel elevation in the expression and activity of the antioxidant enzymes (Jauniaux et al., 2000). On the contrary, the increased concentration of superoxide in the placental tissue of preeclamptic women (Sikkema et al., 2001) was found associated with decreased SOD activity and mRNA expression, for CuZn–SOD in trophoblast cells isolated from preeclamptic placentas (Wang & Walsh, 2001). Also, it was recently found that these women show a decrease in plasma levels of SOD (Aydin et al., 2004). Therefore, a decreased degradation of superoxide anion should be expected. These data are in agreement with another study reporting a decrease in catalase and SOD activities, with a concomitant elevation of lipid peroxidation products (Kumar & Das, 2000). By contrast, in the placenta of women with PE associated with HELLP syndrome, the total glutathione peroxidase activity was more elevated than that in control pregnancies (Kumar & Das, 2000). However, another study reported a decreased activity of glutathione peroxidase, SOD, and glutathione-*S*-transferase in placenta from preeclamptic women (Mutlu-Turkoglu et al., 1998). Although there is some contradiction in the results of studies of the effects of PE on the activity of antioxidant enzymes, ROS formation does appear to be involved. On the other hand, vitamins C and E have demonstrated to increase SOD activity, which decreases superoxide anion concentration in the vascular cells (Chen et al., 2001), a mechanism that could counteract the development of hypertension.

4.2. Nonenzymatic antioxidants

A majority of studies report that women affected by PE have altered levels of natural or endogenous antioxidants in

the blood, placenta, and deciduas (Walsh & Wang, 1993; Bayhan et al., 2000; Kumar & Das, 2000; Zusterzeel et al., 2002; Serdar et al., 2003). Nevertheless, the reduction in antioxidant plasma level does not distinguish women with severe from mild forms of PE (Aksoy et al., 2003). Furthermore, the finding of low concentration of antioxidant vitamins supports the concept of increased oxidative stress in PE. Factors influencing the plasma levels of vitamins C and E in normal and preeclamptic pregnancies will be analysed below.

4.2.1. Vitamin C

Plasma levels of the reduced form of ascorbic acid, a water-soluble vitamin, were reported to be significantly decreased in patients with both mild and severe PE (Kharb, 2000; Mohindra et al., 2002) in association with increased lipid peroxidation (Panburana et al., 2000) and decreased protein thiol levels (Llurba et al., 2004). This effect was attributed to the increased activity of an ascorbate-oxidising system in the plasma of preeclamptic women (Hubel et al., 1997). The reason for the decreased plasma ascorbate is not clear but is consistent with the hypothesis that uteroplacental perfusion induces oxidative stress and ascorbate consumption (Chappell et al., 2002a). The functional consequence and the possible role of decreased plasma ascorbate reserves in PE is consistent with the fact that women who ingested less vitamin C than the recommended daily allowance (85 mg) were at a 2-fold increased risk of developing PE (Zhang et al., 2002).

4.2.2. Vitamin E

During pregnancy, the blood α-tocopherol concentration increases in association with the increase in blood lipid concentration (Horwitt et al., 1972). Newborns have significantly lower plasma vitamin E concentrations than do their mothers (Baker et al., 1975), but when these concentrations are standardised for phospholipids or total lipids, significant differences are not found (Abbasi et al., 1990). Also, it was reported that women with PE show a diminution in placental and maternal plasma levels of the antioxidant carotenoids beta-carotene and lycopene and in placental canthaxanthin (Palan et al., 2001). Furthermore, another study did not find significant differences in plasma α-tocopherol concentration between patients with PE and healthy pregnant women; but α-tocopherol levels corrected by total lipids (cholesterol and triglycerides) showed significantly increased values compared with controls (Llurba et al., 2004). Short-term supplementation of pregnant women before delivery significantly enhanced the vitamin E status of the mother only (Leger et al., 1998), suggesting that vitamin E does not pass efficiently through the placenta to the newborn circulation. A α-tocopherol binding protein was isolated from human placenta, pointing to a role of this protein in regulating the transfer of tocopherols through the placental barrier.

In addition, α-tocopherol and β-carotene levels were found significantly decreased only in severe PE (Mikhail et al., 1994; Sagol et al., 1999; Kharb, 2000). In addition, an inverse correlation between vitamin E levels and lipid peroxidation was reported (El-Salahy et al., 2001), suggesting that vitamin E concentration in plasma may be useful as a prognostic marker of the likely development of PE (Akyol et al., 2000). In addition, there was a relationship found between the extent of vitamin E deficiency and increased lipid peroxidation with increased diastolic blood pressure (Madazli et al., 1999). Thus, decreased plasma levels of vitamin E could be a contributory factor of hypertension in PE.

A majority of studies agree that PE causes a diminution in serum levels of antioxidant vitamins, as well as other lipid soluble antioxidants such as coenzyme Q10 (Palan et al., 2004), carotenoids, and retinol (Williams et al., 2003).

However, other studies did not demonstrate significant differences in plasma vitamin E concentration between women who developed PE and those who did not (Ben-Haroush et al., 2002). Moreover, some results are at variance with the prevailing hypothesis that PE is an antioxidant-deficient state, since increased plasma concentrations of vitamin E among women with PE as compared with normotensive pregnant women have been reported (Uotila et al., 1993; Schiff et al., 1996; Zhang et al., 2001). The latter studies are supportive of the viewpoint that would reject the hypothesis of a causal relationship between decreased dietary vitamin E consumption and plasma levels and the development of PE. These studies suggest that the higher plasma vitamin E concentrations in preeclamptic compared with normal women represent a response to oxidative stress associated with the preeclamptic process, a hypothesis that remains to be demonstrated.

It should be pointed out that the lack of lipid adjustment of plasma vitamin E levels of some studies might explain the controversy between the results. Moreover, plasma vitamin E is a poor index of vitamin E status, as it is lipid bound. Tissue concentrations are actually the only reliable indices. Thus, the plasma tocopherol increase during PE probably reflects the accentuated hyperlipidemia accompanying the disease and confounding the interpretation of plasma vitamin E as indicator of antioxidant status. Therefore, on this basis, it was suggested that if lipid peroxidation is induced in PE, it would be strongly suppressed by the presence of high plasma levels of the lipophylic antioxidant α-tocopherol (Llurba et al., 2004). These authors also suggest that circulating vitamin E levels in PE may be insufficient to protect the increased small, dense low-density lipoprotein (LDL) particles, which result from hypertriglyceridemia and which are particularly susceptible to oxidation (Tribble et al., 1995; Gratacos et al., 2003). Consequently, it could be suggested that vitamin E supplementation to pregnant women at risk of PE would prevent the consequences derived from increased lipid peroxidation.

5. Endothelial dysfunction in preeclampsia

The endothelium is a monolayer of polygonal flat cells that extend continuously over the luminal surface of the entire vasculature, a biological barrier strategically located between the vascular smooth muscle and the blood stream. The functions of the endothelium are numerous and vary according to the size and distribution of blood vessels (Luscher & Barton, 1997). It participates in the modulation of vascular tone, control of primary hemostasis, host defence and inflammation, transport of nutrients, and other solutes, along with the activation and inactivation of various vasoactive hormones. Endothelial dysfunction is characterised by a shift of the actions of the endothelium toward reduced vasodilation, a proinflammatory state, and prothrombic properties (Endemann & Schiffrin, 2004). Generalised endothelial dysfunction could be responsible for all the clinical aspects of the maternal syndrome of PE (Davison et al., 2004). Factors likely to contribute to endothelial dysfunction in PE would be placental ischemia, lipoprotein-induced toxicity, increased ROS, and immune maladaptation, which result in the synthesis and release of proinflammatory cytokines; however, unequivocal data do not exist at this point. Accumulated evidence suggests that oxidative stress is a major mediator of PE, but the underlying mechanism whereby this phenomenon occurs seems to involve many factors, in addition, antiangiogenic modulators are also involved. Placental factors released into the maternal blood stream by the abnormally developed placenta could lead to systemic endothelial dysfunction.

It is known that the enhanced generation of ROS leads to a decrease in NO bioavailability. NO is a humoral factor regulating the foetal placental blood flow, and it maintains low resistance and attenuates the actions of vasoconstrictors in the foetal placental vasculature. In established PE, several suggestions have been advanced to explain the transfer of oxidative stress from the intervillous space to the maternal systemic circulation. Neutrophils and monocytes could be activated by oxidative stress in the intervillous space and then generate the ROS when in contact with the maternal endothelium (Holthe et al., 2004). The transfer of oxidative stress could also be secondary to the formation of stable lipid peroxidation products. It is noteworthy that the augmentation of free radicals could exert important effects in the vascular endothelium (Waktsuki & Okatani, 2000), accounting for the mechanism of vasospasm in PE (Okatani et al., 1997). Numerous mediators have been implicated in the pathophysiology derived from endothelial dysfunction, and lipid peroxides and proinflammatory cytokines are major causal factors (Gratacos, 2000). On the other hand, severe PE is associated with elevated levels of the monocyte chemoattractant protein-1 and interleukin-8 (IL-8). These 2 chemokines are likely induced by a mechanism mediated by oxidative stress that is inhibited by antioxidant treatment with vitamin E (Kauma et al., 2002), highlighting the contribution of oxidative stress to the pathogenesis of endothelial dysfunction involving monocyte recruitment in PE. A more recent study showed that F2-isoprostane mediates the increased IL-8 expression in human macrophages, further supporting a link between oxidative stress/lipid peroxidation and inflammation in these cells and suggesting a role for F2-isoprostanes in this process (Scholz et al., 2003). These aspects of endothelial dysfunction in PE are further discussed below.

5.1. Nitric oxide

During pregnancy, NO is one of the most important relaxing factors of myometrium and also no less important in the control of blood flow in uterus and placenta. It is formed through a reaction catalysed by endothelial NO synthase (eNOS) and plays a major role in maintaining vascular homeostasis. The availability of the cofactor tetrahydrobiopterin (BH_4) is relevant for the enzyme function. In normal placenta, adequate concentrations of L-arginine orient eNOS toward the synthesis of NO. However, in PE, a lower than normal L-arginine concentration caused by arginase II overexpression (Noris et al., 2004) or an alteration in erythrocyte uptake of L-arginine (da Costa et al., 2004) could redirect eNOS toward the synthesis of peroxynitrite. Also, placental hypoxia, which is associated with PE, does not induce an up-regulation of eNOS (Orange et al., 2003). The eNOS inhibitor *N*(omega)-nitro-L-arginine methyl ester (L-NAME) generates a vasoconstrictor response that is significantly decreased in patients with PE, which can be interpreted as a reduced effect of functional NO in the circulation, which proves that the NO pathway is impaired in the vascular bed in these patients (Bisseling et al., 2004). L-NAME administration in pregnant rats induces PE, and the foetuses show endothelial dysfunction that disappears after birth (Martinez-Orgado et al., 2004).

Because the placenta lacks autonomic innervation, locally produced mediators like NO play an important role in the maintenance of placental blood flow. In addition, NO regulates leukocyte adhesion to the endothelium (Kubes et al., 1991) and inhibits vascular smooth muscle cell proliferation and platelet aggregation (Radomski et al., 1987). All these functions could be impaired by the consumption of NO by superoxide anions produced during oxidative stress, thereby diminishing NO bioavailability. Consistent with this was the finding of a diminished concentration of NO in plasma (Var et al., 2003b; Aydin et al., 2004) or amniotic fluid (Tranquilli et al., 2004) in preeclamptic women. A study performed in an ex vivo cotyledon perfusion model in women with PE demonstrated that the antioxidant *N*-acetylcysteine improves the endothelial function mediated by the NO pathway, supporting the view that the impairment in this pathway in PE is related to the occurrence of oxidative stress (Bisseling et al., 2004). Recently, it was found that increased NO production correlated well with an increased plasma level of neurokinin

B, a neuropeptide that belongs to the tachykinin family, suggesting that this response may be a compensatory mechanism to improve blood flow to the uteroplacental unit (D'Anna et al., 2004). However, because the spiral arteries are unresponsive and cannot be dilated, an adequate blood supply to the placenta fails to occur; therefore, raised levels of neurokinin B may then lead to maternal hypertension and damage the kidneys and liver. The role of neurokinin B in PE remains to be determined (Schlembach et al., 2003).

Despite the numerous studies addressed to examine NO production, assessed by monitoring plasma levels of the NO stable degradation products nitrites and nitrates (NOx) in both normal pregnancy and PE, the status of NO is controversial. Thus, an elevation (Nobunaga et al., 1996; Smárason et al., 1997), a decrease (Seligman et al., 1994; Begum et al., 1996; Davidge et al., 1996; Choi et al., 2002), or no change (Silver et al., 1996; Diejomaoh et al., 2004) has been shown in NOx levels. This controversy may be partly due to the effect of potential confounders. In this regard, it should be mentioned the relevance of the control in the dietary intake of nitrate, which can dramatically affect the plasma level and urinary excretion of NOx. In addition, the plasma levels of NOx are influenced by acute fluctuations in their renal tubular reabsorption (Conrad et al., 1999). The authors used a reduced NOx diet prior to analyse the plasma and urinary levels of NOx and its second messenger cGMP. Despite increases in both plasma and urinary cGMP during normal human pregnancy, NOx were either unchanged or reduced, suggesting that (1) another signal besides NO mediates augmented cGMP production and maternal vasodilation during pregnancy, or (2) body fluid NOx is an unreliable estimate of hemodynamically relevant NO. In turn, in preeclampsia, unequivocal support for reduced NO production was not demonstrated.

Recently, it was reported that decreased eNOS expression and activity may be associated with increased endothelial permeability in PE (Wang et al., 2004). These authors suggested that IL-8, which is increased by oxidative stress (Scholz et al., 2003), could be a candidate agent that mediates this effect. The inhibition of eNOS resulted in enhanced IL-8-induced endothelial monolayer permeability in preeclamptic endothelial cells compared with those from normal pregnancies, probably due to a higher susceptibility to proinflammatory cytokines in PE; however, these data need to be confirmed. Finally, modulation of NOS isoforms by endothelin (ET)-1, as found in PE, is suggestive of a functional relationship. Thus, trophoblastic cells in preeclamptic women show an increased expression of eNOS, whereas that of the inducible isoform (iNOS) being the main source of NO synthesis, is decreased (Napolitano et al., 2000).

5.2. Asymmetric dimethylarginine

The generation of NO by eNOS could be down-regulated in PE by hypoxia and by increased levels of asymmetric dimethyl arginine (ADMA), as well as by a diminished enzyme expression. An elevated ADMA concentration has been attributed to hypertension (Surdacki et al., 1999), hyperlipidemia (Boger et al., 1998), and hyperhomocysteinemia (Sydow et al., 2003; Stuhlinger et al., 2003), 3 alterations likely to be found in PE. Accordingly, ADMA has been reported to be elevated in the plasma of women with PE (Holden et al., 1998), which could directly interfere with NO and induce endothelial dysfunction, due to its ability to behave as an endogenous competitive inhibitor of eNOS.

Elevated circulating ADMA concentrations, in combination with low plasma arginine levels, have been suggested to be of pathophysiological importance in pregnancies complicated with PE (Pettersson et al., 1998). In addition, ADMA causes an uncoupling, where the activity of the enzyme for NO production is decreased in association with an increase in eNOS-dependent superoxide anions formation (Touyz & Schiffrin, 2004). Women at risk of PE, having a high resistance in the placental circulation, a foetus with a low weight for gestational age, or both, show elevated concentrations of ADMA, which is a potential contributory factor for the development of PE and is associated with endothelial dysfunction (Savvidou et al., 2003). Angiotensin-converting enzyme inhibitors and AT1 receptor blockers can diminish ADMA generation, otherwise stimulated by angiotensin II (Delles et al., 2002), but the latter mechanism is not well understood.

Recently, the association of increased plasma levels of ADMA and PE has been called into question by a study reporting no significant differences between preeclamptic and normal pregnant women (Maas et al., 2004). The authors also showed lack of ethnicity-related differences in ADMA concentration in White, African, indigenous, and multiethnic pregnant women, thus suggesting that preeclampsia in low- and high-risk populations may have distinct underlying causes.

5.3. Peroxynitrite

Peroxynitrite can alter cellular functions in many ways and nitrate many proteins, particularly tyrosine moieties. In addition, peroxynitrite diminishes NO bioavailability because it oxidises BH_4, a cofactor for eNOS, thereby leading to degradation of the enzyme (Milstien & Katusic, 1999; Szabo et al., 2003), an effect that could be blunted by vitamin C (Baker et al., 2001). The altered vascular reactivity occurring in the preeclamptic placenta may be partly explained by the peroxynitrite effect inducing vascular dysfunction via selective impairment of adrenoreceptors (Benkuski et al., 1999). It has been demonstrated that peroxynitrite also selectively inactivates the prostacyclin receptor (Zou et al., 1999) and decreases the amount of prostacyclin synthase through a mechanism mediated by NF-κB (Cooke & Davidge, 2002). These peroxynitrite effects contribute to the development of hypertension in

PE. Furthermore, cytotrophoblasts from preeclamptic women had an increased thromboxane production, thereby causing an imbalance to the thromboxane and prostacyclin production (Ding et al., 2002), further contributing to a generally observed elevated blood pressure.

5.4. Endothelin and F2-isoprostanes

The biological effects of F2-isoprostanes include a potent vasoconstriction in the kidney, lung, heart, brain, and placenta (McGiff & Quilley, 2001). The underlying mechanism of these effects may occur through a stimulation of IP3 and mitogenesis in vascular smooth muscle cells (Fukunaga et al., 1993) and a release of endothelin-1 from endothelial cells (Fukunaga et al., 1995). Indeed, ROS-generated F2-isoprostanes stimulate DNA synthesis and endothelin-1 expression in endothelial cells (Yura et al., 1999). Accordingly, it was proposed that these compounds may mediate in the development of hypertension, as found in preeclamptic patients, because the increased placental secretion of F2-isoprostanes into the maternal circulation could cause vasoconstriction in maternal vascular beds (Walsh et al., 2000). In addition, it was demonstrated that F2-isoprostanes released from the preeclamptic decidua basalis are significantly elevated at delivery (Staff et al., 1999).

5.5. Homocysteine

Elevated homocysteine (Hcy) concentrations are associated with impaired endothelium-dependent vasodilatation and early manifestation of atherosclerosis. Lowering plasma Hcy concentrations with vitamin B treatment is associated with improved vascular endothelial function. Elevated levels of reduced Hcy promote endothelial injury and react with NO in the presence of oxygen to form *S*-nitrosohomocysteine, which may decrease the bioactivity of NO, due to newly formed and stable nitrosothiol (Chambers et al., 2001). This reduced form of Hcy promotes the generation of oxygen-derived free radicals via an increase in oxidised LDL (oxLDL). Although the Hcy concentration falls during normal pregnancy (Hague, 2003; Powers et al., 2004), increased plasma levels have been observed in ~20–30% of women with PE. In addition, hyperhomocysteinemia is reported to be associated with PE (Var et al., 2003b; D'Anna et al., 2004; Raijmakers et al., 2004b), which positively correlates with MDA levels (Tug et al., 2003), the pressure profile (Noto et al., 2003), intrauterine growth restriction (D'Anna et al., 2004), and the level of soluble vascular cell adhesion molecule 1 (VCAM-1; Vadachkoria et al., 2004). On the other hand, increased Hcy levels are closely associated with decreased NO (Var et al., 2003b). Also, Hcy induces trophoblast apoptosis and significantly reduces human chorionic gonadotropin secretion (Di Simone et al., 2003).

The mechanism for endothelial dysfunction in hyperhomocysteinemia has not been elucidated. Nevertheless, it could be partly explained on the basis of a diminution of NO bioavailability leading to oxidative stress (Rodrigo et al., 2003). It is interesting to note here that in a pharmacological attempt to control hyperhomocysteinemia in men, given a daily vitamin supplement (10 mg pyridoxal, 1.0 mg folic acid, 0.4 mg cyanocobalamin, with a placebo-controlled follow-up study), normalised elevated plasma concentrations of Hcy were apparent within 6 weeks (Ubbink et al., 1993). A hypothesis to explain the generation of hypertension on the basis of Hcy-related contributory factors is depicted in Fig. 1.

5.6. Dyslipidemia

Early pregnancy dyslipidemia has been reported to be associated with an increased risk of PE. Hypertriglyceridemic dyslipidemia before 20 weeks of gestation is associated with the risk of developing early but not late-onset PE (Clausen et al., 2001). An increase in the risk of PE was observed with increasing levels of LDL cholesterol, triglycerides concentrations, and LDL/high-density lipoprotein (HDL) ratio (Enquobahrie et al., 2004). Indeed, triglycerides and free fatty acids are already elevated in the first and the second trimesters in these cases (Endresen et al., 1994; Gratacos et al., 1996). In addition, several studies reported high plasma levels of atherogenic small dense LDL in women with PE (Sattar et al., 1997; Hubel et al., 1998), which are susceptible to oxidative modification and can exert deleterious effects on the endothelium, a result not found in Black women (Patrick et al., 2004). A single injection of native LDL in rats causes a marked decrease in the vasodilator response to acetylcholine concomitantly with an increase in the levels of ADMA and MDA. However, pretreatment with 17 β-estradiol significantly attenuates the inhibition of this vasodilator response and the elevation of both ADMA and MDA concentration. The authors suggest that estradiol possesses a protective effect on the endothelium related to reduction of ADMA concentration by the inhibition of lipid peroxidation (Dai et al., 2004). PE could thus represent an acute model of endothelial cell activation with subsequent effects of lipid peroxides in combination with other endothelial and proinflammatory stimuli. Although the lipid aberrations seen in PE may appear unrelated, they have all been suggested to contribute to a causative role of endothelin-1 in the aetiology of PE (Coffey, 2003). Hyperlipidemia in pregnancy has been suggested to be a promoter of endothelial dysfunction, probably by inducing oxidative stress in the arterial wall. This effect is thought to be exacerbated in PE by the combination of lipoproteins and placental derived factors, such as lipid peroxides and trophoblastic components, forming complexes more deleterious to endothelial cells than the individual effects (Lorentzen & Henriksen, 1998). It deserves special mention that oxLDL formed by increased oxidative stress status, but not native LDL, inhibit human trophoblast cell invasion in a concentration-dependent

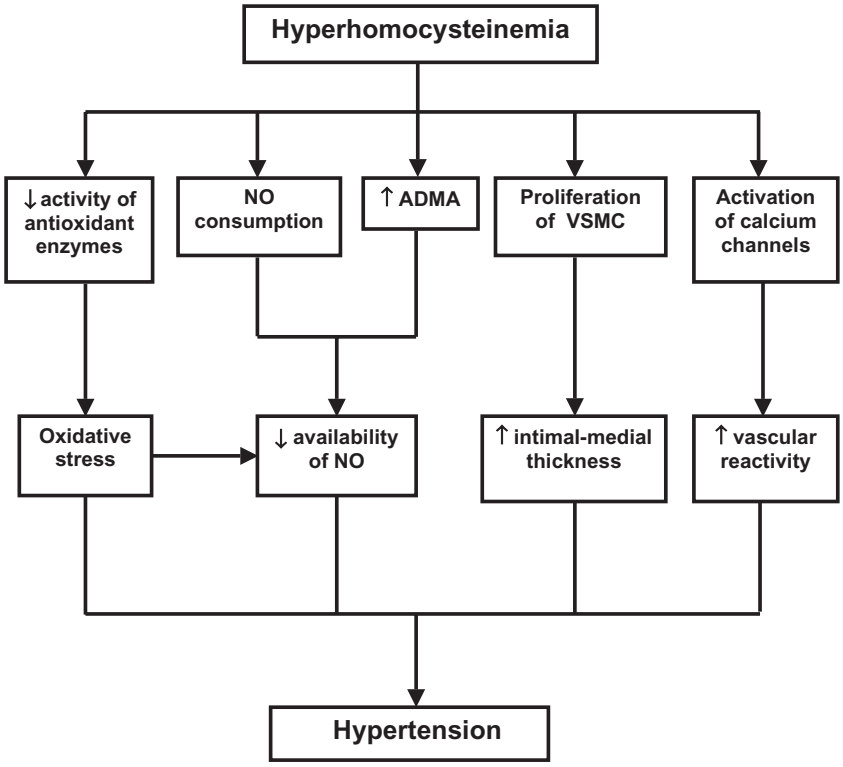

Fig. 1. Schematic diagram illustrating the proposed role of hyperhomocysteinemia in the development of hypertension in preeclamptic women. ADMA, asymmetric dimethyl arginine; NO, nitric oxide; VSMC, vascular smooth muscle cells.

manner, suggesting that the process of invasion may be modulated by oxLDL in vivo (Pavan et al., 2004). This view provides new insights into the pathophysiology of PE associated with oxidative stress and defective trophoblast invasion.

5.7. Nuclear factor-kappa B

Nuclear factor kappa-B (NF-κB) is a transcription factor involved in enhancing the expression of various proinflammatory biochemical markers including cytokines, chemokines, and cellular adhesion molecules (Baeuerle & Henkel, 1994; Baldwin, 1996). The first step in this cascade is the activation of cytoplasmic inactive dimers of NF-κB, which are bound to I-κB. I-κB is phosphorylated, ubiquitinated, and ultimately degraded by proteasomes. Unbound dimers so formed are translocated into the nucleus, bind to DNA, and activate inflammatory genes. It was demonstrated that women with severe PE possessed up-regulated NF-κB in HUVEC. By contrast, NF-κB activity is suppressed in peripheral mononuclear cells from pregnant women, a condition exacerbated in PE (McCracken et al., 2003). Factors released from preeclamptic trophoblast cells significantly increase the expression in HUVEC of markers of endothelial cell activation, such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), P-selectin, and E-selectin, all causing some remarkable pathophysiological effects of PE, possibly through the activation of NF-κB (Wang et al., 2003). The increased expression of ICAM-1, paralleled by the up-regulation of NF-κB, is blunted by antioxidant treatment with both vitamin E and *N*-acetylcysteine (Takacs et al., 2001), thus supporting the involvement of oxidative stress in endothelial dysfunction. In turn, the increased expression of P-selectin and E-selectin in HUVEC cocultured with PE trophoblast cells is significantly attenuated by chymotrypsin inhibition, suggesting that this enzyme participates in the selectin up-regulation in this disease (Wang et al., 2003).

6. Angiogenesis-related factors

Due to the fact that the angiogenic balance could be impaired in PE, considerable attention has recently been focused on angiogenesis-related gene products, including vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and soluble fms-like tyrosine kinase 1 (sFlt1). VEGF is an endothelial-specific mitogen, which plays a key role in promoting angiogenesis, as seen in a variety of cells, including the endothelial cells at the vasculature of the placenta. VEGF induces mitosis in the endothelium and is required for the cells to survive prolonged periods and function properly. PlGF is another member of the VEGF family that is predominantly made in the placenta. sFlt1 is an endogenously secreted protein that captures free VEGF and PlGF, thus preventing these molecules from interacting with the endogenous receptor. In preeclampsia, free VEGF and PlGF synthesis is reduced,

whereas sFlt1 is up-regulated in the placenta from the second trimester of pregnancy (Maynard et al., 2003).

ROS derived from the NADPH oxidase complex are critically important in many aspects of vascular cell regulation. In this regard, a role of NADPH oxidase in VEGF-induced angiogenesis has been suggested: NADPH oxidase-derived ROS was suggested to participate in VEGF signalling and angiogenesis-associated responses in vitro and in vivo. Because angiogenesis is tightly controlled by the reduction/oxidation environment at the level of the VEGF receptor, it is possible that the up-regulation of VEGF is mediated by ROS (Maulik & Das, 2002), thus providing a novel potential therapeutic target for angiogenesis-dependent diseases. This view is supported by several studies. First, VEGF induces a significant increase in superoxide production, as shown by electron spin resonance with spin probe cyclic hydroxylamine PP-H (Dikalov et al., 2002). Second, the functional role of NADPH oxidase activity for this response was demonstrated in cultured endothelial cells using chemical inhibitors of the enzyme (Abid et al., 2000). Third, it was demonstrated that the VEGF-dependent ROS stimulation occurs via a Rac1-dependent, $gp91^{phox}$-containing NADPH oxidase that is essential to cell migration and proliferation of HUVEC (Ushio-Fukai et al., 2002). In summary, VEGF stimulates superoxide production in HUVEC through the activation of $Rac1^{-}$ and $gp91^{phox}$, 2 components of NADPH oxidase complex of endothelial cells. Therefore, it should be expected an impaired angiogenesis in PE, due to the diminution of free VEGF induced by the up-regulation of the expression of placental sFlt1 that prevents the interaction of VEGF with the endogenous receptor. More recent studies have revealed that angiogenesis assessed through endothelial cell migration and in vitro tube formation was attenuated by preincubation with exogenous sFlt1, and this effect was abolished by the removal of sFlt1 by immunoprecipitation (Ahmad & Ahmed, 2004). It has been hypothesised that the elevated level of sFlt1 detected in placenta from women with preeclampsia is a consequence of the placental hypoxia that occurs during abnormal placentation (Karumanchi & Bdolah, 2004). Recently, this view has gained support from studies performed in explants of placenta and cultured placental cells. Normal placental villous explants exposed to hypoxic conditions that mimics oxygen tension of placenta from women with PE show significantly increased production of sFlt1, compared with tissue normoxia (Ahmad & Ahmed, 2004). This effect is mediated by hypoxia-induced factor (HIF-1α) binding to a hypoxia response element in the flt1 gene promoter (Gerber et al., 1997). Accordingly, it was demonstrated that reduced oxygen resulted in a pronounced increase in sFlt1 mRNA amount and sFlt1 release into the culture media in cytotrophoblasts, whereas this was not the case with HUVEC and fibroblasts (Nagamatsu et al., 2004). Despite that VEGF expression is stimulated by reduced oxygen concentration, free VEGF could not be detected in these cytotrophoblast culture media,

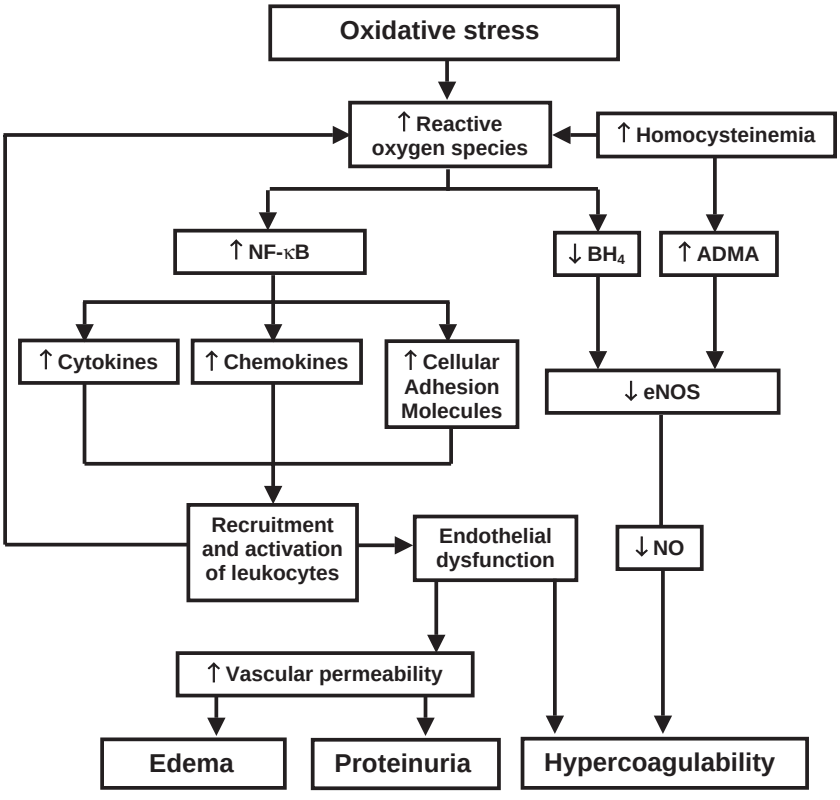

Fig. 2. Schematic diagram illustrating the main contributory factors involving endothelial dysfunction that underlies the clinical feature of PE. ADMA, asymmetric dimethyl arginine; BH_4, tetrahydrobiopterin; eNOS, endothelial nitric oxide synthase; NF-κB, nuclear factor kappa B; NO, nitric oxide.

similar to the stimulation in HUVEC and fibroblasts. These data provide new evidence that the effects of placental hypoxia are cell type specific and that cytotrophoblasts possess a unique property in that they enhance their ability to produce sFlt1, which could antagonise the angiogenic activity of VEGF and PlGF.

The subsequent endothelial dysfunction may disrupt the blood–brain barrier and cause edema in the liver and may also affect glomerular function, thus accounting for the derangement known to occur in the disease (Maynard et al., 2003). In addition, in the second trimester of preeclamptic pregnancies, decreased plasma levels of VEGF have been found to be associated with a diminution of NO. This has been suggested to represent an impaired stimulus to vascular formation and endothelial regulation that induces placental disease and PE (Tranquilli et al., 2004). The finding that sFlt1 administration to mice induces a PE-like phenotype highlights its role in the pathophysiology of PE. Also, anti-VEGF antibody administration causes rapid proteinuria, which is associated with a disruption of glomerular endothelial cells (Sugimoto et al., 2003). VEGF also induces vasodilation, although it is not a powerful effect, hence it could be expected that the decreased levels of VEGF could contribute in some extent to the elevated blood pressure. This could explain several, although not all, of the major symptoms of PE (Luttun & Carmeliet, 2003). On the basis of these data, a hypothesis was recently put forward that an elevated sFlt1 is not so much a consequence of the maternal syndrome but it may, in fact, induce it (Davison et al., 2004). Thus, an increased level of sFlt1 was suggested as a biomarker for the subsequent development of PE (Levine et al., 2004). Nevertheless, although the serum levels of sFlt1 in the women with PE begin to escalate approximately by gestational age of 20 weeks, they are not significantly higher than that of the controls until the onset of the clinical disease.

A summary of the main contributory factors involving endothelial dysfunction that underlie the clinical features of PE is shown in Figs. 2 and 3.

7. Antioxidant therapy in the prevention of preeclampsia: vitamins C and E

Antioxidant therapy of PE is based on the paradigm that the overproduction of ROS contributes to the endothelial cell activation accounting for the clinical expression of the disease. On this basis, it could be suggested that the therapeutic use of vitamins C and E might prevent these complications of pregnancy. Accordingly, Chappell et al. (1999), in a randomised trial of 283 women at high risk for PE, showed a reduction in the incidence of the disease from 17% in the placebo group to 8% in the group treated with vitamins C and E from the second trimester of pregnancy. This effect was accompanied by a diminution of the indices of oxidative stress towards values that were observed in a group of healthy women (Chappell et al., 2002b). In contrast, some authors have called into question the importance of oxidative stress in PE and the biochemical rationale for clinical trials of antioxidants to prevent and

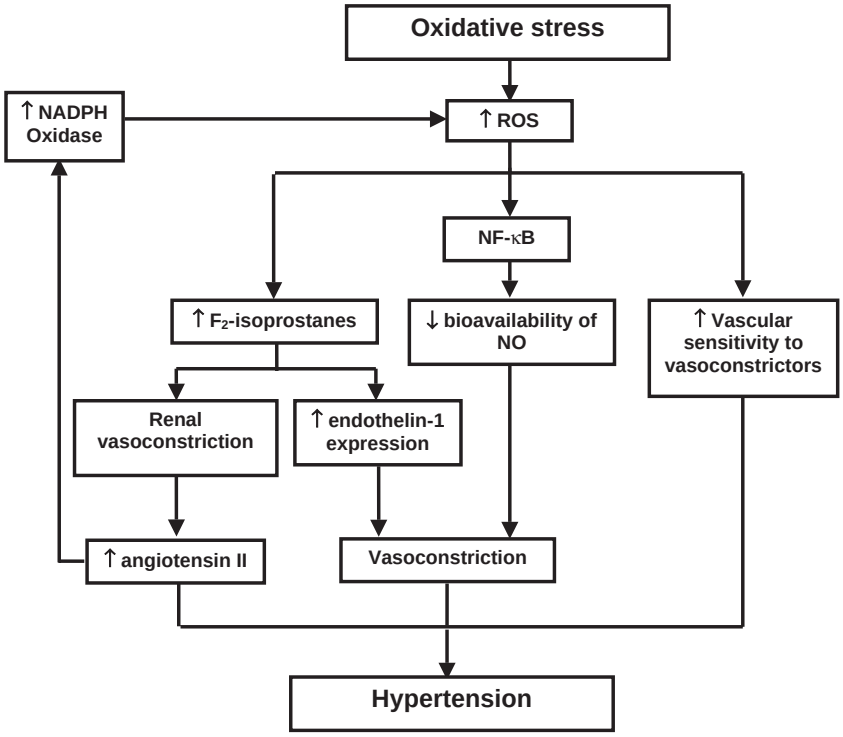

Fig. 3. Schematic diagram illustrating the proposed role of oxidative stress in the development of hypertension in preeclamptic women. NF-κB, nuclear factor kappa B; NO, nitric oxide; ROS, reactive oxygen species.

treat the disease (Regan et al., 2001). The latter studies found no evidence of increased lipid peroxidation in preeclamptic women, based on the determination of diverse urinary cyclooxygenase-independent isoprostanes. However, these measurements excluded F2-isoprostanes, widely accepted as reliable markers of lipid peroxidation (Liu et al., 1998). Although F2-isoprostanes also could be enzymatically generated, via cyclooxygenase-2, their augmented placental secretion in PE is paralleled by increased MDA production, with a correlation of 0.98 (Walsh et al., 2000), which is a more convincing evidence of the occurrence of placental oxidative stress than the abovementioned urinary determinations. Attempts to characterise gestational profiles of biochemical markers that are associated with preeclampsia in the blood of pregnant women in whom preeclampsia developed later have been done (Chappell et al., 2002a). The mechanisms whereby vitamins C and E could attenuate oxidative stress and endothelial dysfunction, thereby supporting a prophylactic effect, will be discussed below.

7.1. Modulation of antioxidant status

Vitamins C and E are 2 essential nutrients that can scavenge free radicals and constitute a strong line of defence in retarding ROS-induced cellular damage. Moreover, it has been demonstrated a synergistic effect between the 2 vitamins in vitro and in vivo (Salonen et al., 2000). Vitamin E, mainly α-tocopherol, is the major peroxyl radical scavenger in biological lipid phases, such as membranes or LDL. Its antioxidant action has been ascribed to its ability to chemically act as a lipid-based free radical chain-breaking molecule, thereby inhibiting lipid peroxidation and oxLDL formation. During normal pregnancy, plasma vitamin E concentrations show a progressive elevation, what could be due to the gestational increase in circulating lipoproteins, the transporters of vitamin E. In patients with mild PE, maternal blood α-tocopherol concentrations are not decreased compared with normal pregnancies (Mikhail et al., 1994; Sagol et al., 1999), but in patients with severe PE, plasma α-tocopherol was significantly decreased compared with controls, which is thought to be caused by the fact that antioxidants may be utilised to a greater extent to counteract free radical-mediated cell disturbances, resulting in a reduction in their plasma levels (Kharb, 2000). On the other hand, plasma levels of ascorbate decrease gradually throughout normal pregnancy (Mikhail et al., 1994), but in PE patients, the reserves are decreased by 20–50% over normal pregnancy levels (Zhang et al., 2002). Ascorbic acid has been shown to scavenge free radicals directly in the aqueous phases of cells and the circulatory system. However, it has also been proven to protect membrane and other hydrophobic compartments from damage by regenerating the antioxidant form of vitamin E. Thus, it has been established that ascorbic acid can support the antioxidant activity of α-tocopherol by reducing tocopheroxyl radicals directly or indirectly, thus acting to regenerate oxidised vitamin E to the reduced form by nonenzymic mechanism (Chan, 1993). The proposed mechanism of action was that when vitamin E intercepts a radical, α-tocopheroxyl radical is formed, which can be reduced back to α-tocopherol by vitamin C or other reducing agents, thus attenuating the propagation of free radical reactions (Zhang et al., 2002). Thus, vitamin C prevents the prooxidant activity of vitamin E by decreasing the activity of α-tocopheroxyl radical to α-tocopherol, thereby contributing to increased total antioxidant status and reduced oxidative stress (Chen et al., 2001). Taken together, these data would support the hypothesis that supplementation with vitamins C and E would prevent the clinical consequences derived from oxidative stress in PE.

7.2. Modulation of endothelial cell function

Although vitamins C and E have been mislabelled as simply biological antioxidants, they also could play a key role in the modulation of enzymes involved in the vascular endothelial damage known to contribute to the pathophysiological mechanism of the clinical expression of PE. As was previously discussed in the present review, in the vascular cells, NADPH oxidase is the major source of superoxide anion, a key mediator in the development of systemic pathological processes such as impairment of endothelium-dependent vasodilatation, inflammation or increased platelet aggregation. The activity of NADPH oxidase is directly down-regulated by vitamins C and E (Ülker et al., 2003), thereby contributing to counteract the development of these derangements in PE. These data are in agreement with previous studies demonstrating that both vitamins inhibit superoxide anion production in pig coronary artery (Nunes et al., 1997). In addition, in preparations of aorta from spontaneously hypertensive rats, it was found that vitamins C and E, separately, caused an enhancement of the activity of eNOS and NO generation (Ülker et al., 2003), an effect expected to ameliorate the deleterious vascular consequences derived from endothelial dysfunction. The mechanisms whereby vitamins C and E may cause down-regulation of NADPH oxidase and up-regulation of eNOS are unclear, but it has been suggested that they may play an important role in the regulation of protein expression of the NADPH oxidase at the transcriptional or post-translational levels (Chaudiere & Ferrari-Iliou, 1999). In addition, we should not discard the possibility that vitamins C and E directly influence the biological activity of the enzymes. Vitamin E is located within the cell membrane and could alter cell membrane-associated NADPH oxidase by inhibiting or interrupting the complex formation of the NADPH oxidase subunits (Chen et al., 2001). Accordingly, α-tocopherol has been shown to inhibit the activation of NADPH oxidase by prevention of $p47^{phox}$ membrane translocation and phosphorylation (Cachia et al., 1998). Furthermore, another beneficial effect on endothelial cell function results from

the ability of vitamins C and E to stimulate the activity of eNOS by increasing the intracellular availability of the eNOS cofactor BH_4, which would further increase NO synthesis (Taddei et al., 1998; Newaz et al., 1999), thus enhancing this enzyme activity (Baker et al., 2001). In addition, vitamin C treatment improves endothelial NO action, increases prostacyclin production, and lowers blood pressure in coronary patients (Moran et al., 1993; Nunes et al., 1997; Vita et al., 1998). Finally, the beneficial effects of vitamin E in endothelial cell function also have been related with its ability to inhibit the up-regulation of ICAM-1 (Takacs et al., 2001) and the increased production of IL-6 (Takacs et al., 2003), 2 effects of PE thought to be mediated by NF-κB activation.

7.3. Prophylactic use in preeclampsia

Although it is uncertain that oxidative stress be the primary event in the mechanism of PE, it plays a major role in the pathophysiology of the disease, highlighting the benefit of an antioxidant therapy as a mean to counteract the deleterious effects of ROS. Normal pregnancy per se is accompanied by increased indices of oxidative stress, but further elevation of these indices, as well as those of endothelial dysfunction, are found in PE after 20 weeks of gestation (Chappell et al., 2002b). Because the appearance of abnormal uterine artery Doppler flow velocity waveforms, a characteristic feature of PE, coincides with the onset of the established PE, an antioxidant therapy given later would be of doubtful efficacy. Therefore, it could be recommended to initiate this prophylaxis before the 20th week of gestation, after identifying the risk factors on the basis of the medical history of the patient, what would lower the risk of maternal vascular dysfunction and therein the onset of PE. The rationale for the recommendation of vitamins C and E supplementation towards the prevention of PE concerning the oxidative events was recently reviewed (Bilodeau & Hubel, 2003; Raijmakers et al., 2004a). Supplements designed for pregnancy usually contain small doses of α-tocopherol; however, no adverse effects have been observed with higher levels of supplementation (Brigelius-Flohe et al., 2002). Furthermore, short-term supplementation of pregnant women before delivery significantly enhanced the vitamin E status of the mother only, suggesting that vitamin E does not pass efficiently through the placenta to the newborn circulation. Despite the promising role for vitamins C and E in preventing pregnancy complications, very little is known about their maternal and foetal pharmacodynamics (Pressman et al., 2003). However, the lack of maternal and foetal adverse effects reported for vitamins C and E in pregnancy at doses known to reduce the oxidative stress and its consequences raises the possibility of their administration to pregnant women without involving foetal and maternal risk. Finally, it should be noted that hyperhomocysteinemia is associated with the occurrence of oxidative stress and endothelial dysfunction. Therefore, in these cases, the amelioration of vascular impairment found in PE through vitamins C and E supplementation could be reinforced by inducing also a diminution of homocysteinemia, what could be reached by the addition of vitamins B12 and B6 plus folic acid (Ubbink et al., 1993).

8. Concluding remarks and perspectives

PE, a disorder secondary to decreased placental perfusion, which involves various maternal constitutional factors and cell dysfunction, is broadly associated with endothelial activation. Despite much research, controversy remains as to the importance of oxidative stress as the underlying mechanism of endothelial dysfunction in PE, although the majority of studies point to its occurrence from the third trimester. The antioxidant defence system would be expected to counteract the deleterious effect of oxidative stress, however, the expression of the main antioxidant enzymes and other antioxidant systems are reported decreased in preeclamptic placental tissues and in the plasma, with a concomitant increase in lipid peroxidation products. Additionally, although the possible role of sFlt1 as a preeclamptic factor is just emerging, it seems likely that other pathophysiological events occur prior its increased placental production. The fact that radical-scavenging antioxidants are consumed by increased free radical activity in PE has provided a rational basis for potential therapeutic interventions based on the early administration of antioxidants to pregnant women at risk. Studies of the role of vitamin C and E in the prevention of PE have focused on its antioxidant properties, but their biological effects surpass their ability as antioxidant molecules. Thus, vitamins C and E also prevent the impairment of endothelial cell function, among other non-antioxidant mechanisms. Accordingly, the prophylactic supplementation of vitamins C and E to pregnant women is associated with reduced incidence of PE and decreased placental and endothelial dysfunction. Recently, a combination of vitamins C and E has been recommended as a promising prophylactic strategy for prevention of PE, although further studies are still lacking. In addition, a diminution of hyperhomocysteinemia to normal plasma levels has been obtained within 6 weeks through supplementation with a mixture of vitamins B6, B12, and folic acid. These interventions are consistent with the view that ascorbic acid and α-tocopherol behave not only as ROS scavengers, but also as enzyme modulators, thus avoiding superoxide anion formation and increasing the bioavailability of NO. Thus, it should be expected that this therapeutic intervention will decrease the morbidity and mortality associated with PE (in addition to decreasing PE incidence), although it is premature to ensure that the dosages (e.g., 1 g ascorbate and 400 IU tocopherol daily) are safe with respect to foetal development. Nevertheless,

it can be concluded that there is relevance for the application of large-scale multicenter clinical trials so as to draw more definite conclusions. These studies, accompanied by early diagnostic recognition, would contribute to new hope that novel strategies based on a pharmacological prophylaxis of PE could reduce the incidence of PE in high-risk groups. Thus, supplementation with vitamins may be designed as a therapeutic opportunity that would combat PE and thereby improve the healthy outcome for both the mother and child.

Acknowledgments

This study was supported by the Fondo Nacional de Investigación Científica y Tecnológica (FONDECYT), grants 1020080 and 1040429.

References

Abbasi, S., Ludomirski, A., Bhutani, V. K., Weiner, S., & Johnson, L. (1990). Maternal and fetal plasma vitamin E to total lipid ratio and fetal RBC antioxidant function during gestational development. *J Am Coll Nutr 9*, 314–319.

Abid, M. R., Kachra, Z., Spokes, K. C., & Aird, W. C. (2000). NADPH oxidase activity is required for endothelial cell proliferation and migration. *FEBS Lett 486*, 252–256.

Afifi, Y., & Churchill, D. (2003). Pharmacological treatment of hypertension in pregnancy. *Curr Pharm Des 9*, 1745–1753.

Akyol, D., Mungan, T., Gorkemli, H., & Nuhoglu, G. (2000). Maternal levels of vitamin E in normal and preeclamptic pregnancy. *Arch Gynecol Obstet 263*, 151–155.

Aksoy, H., Taysi, S., Altinkaynak, K., Bakan, E., Bakan, N., & Kumtepe, Y. (2003). Antioxidant potential and transferrin, ceruloplasmin, and lipid peroxidation levels in women with preeclampsia. *J Invest Med 51*, 284–287.

Ahmad, S., & Ahmed, A. (2004). Elevated placental soluble vascular endothelial growth factor receptor-1 inhibits angiogenesis in preeclampsia. *Circ Res 95*, 884–891.

Aquilina, J., & Harrington, K. (1996). Pregnancy hypertension and uterine artery Doppler ultrasound. *Curr Opin Obstet Gynecol 8*, 435–440.

Aydin, S., Benian, A., Madazli, R., Uludag, S., Uzun, H., & Kaya, S. (2004). Plasma malondialdehyde, superoxide dismutase, sE-selectin, fibronectin, endothelin-1 and nitric oxide levels in women with preeclampsia. *Eur J Obstet Gynecol Reprod Biol 113*, 21–25.

Bae, I., Fan, S., Meng, Q., Rih, J. K., Kim, H. J., Kang, H. J., et al. (2004). BRCA1 induces antioxidant gene expression and resistance to oxidative stress. *Cancer Res 64*, 7893–7909.

Baeuerle, P. A., & Henkel, T. (1994). Function and activation of NF-kappa B in the immune system. *Annu Rev Immunol 12*, 141–179.

Baker, H., Frank, O., Thomson, A. D., Langer, A., Munves, E. D., De Angelis, B., et al. (1975). Vitamin profile of 174 mothers and newborns at parturition. *Am J Clin Nutr 28*, 59–65.

Baker, T. A., Milstien, S., & Katusic, Z. S. (2001). Effect of vitamin C on the availability of tetrahydrobiopterin in human endothelial cells. *J Cardiovasc Pharmacol 37*, 333–338.

Baldwin Jr, A. S. (1996). The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol 14*, 649–683.

Barden, A., Beilin, L. J., Ritchie, J., Croft, K. D., Walters, B. N., & Michael, C. A. (1996). Plasma and urinary 8-*iso*-prostane as an indicator of lipid peroxidation in pre-eclampsia and normal pregnancy. *Clin Sci (Lond) 91*, 711–718.

Barden, A., Ritchie, J., Walters, B., Michael, C., Rivera, J., Mori, T., et al. (2001). Study of plasma factors associated with neutrophil activation and lipid peroxidation in preeclampsia. *Hypertension 38*, 803–808.

Basbug, M., Demir, I., Serin, I. S., Ozcelik, B., Saraymen, R., Narin, F., et al. (2003). Maternal erythrocyte malondialdehyde level in preeclampsia prediction: a longitudinal study. *J Perinat Med 31*, 469–474.

Bayhan, G., Atamer, Y., Atamer, A., Yokus, B., & Baylan, Y. (2000). Significance of changes in lipid peroxides and antioxidant enzyme activities in pregnant women with preeclampsia and eclampsia. *Clin Exp Obstet Gynecol 27*, 142–146.

Beckman, J. S., & Koppenol, W. H. (1996). Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol 271*(5 Pt 1), C1424–C1437.

Begum, S., Yamasaki, M., & Mochizuki, M. (1996). The role of nitric oxide metabolites during pregnancy. *Kobe J Med Sci 42*, 131–141.

Ben-Haroush, A., Harrell, D., Hod, M., Bardin, R., Kaplan, B., Orvieto, R., et al. (2002). Plasma levels of vitamin E in pregnant women prior to the development of preeclampsia and other hypertensive complications. *Gynecol Obstet Invest 54*, 26–30.

Benkuski, N. A., Lewis, S. J., & Kooy, N. W. (1999). Peroxynitrite-mediated attenuation of alpha- and beta-adrenoreceptor against-induced vascular responses in vivo. *Eur J Pharmacol 364*, 151–158.

Bilodeau, J. F., & Hubel, C. A. (2003). Current concepts in the use of antioxidants for the treatment of preeclampsia. *J Obstet Gynaecol Can 25*, 742–750.

Bisseling, T. M., Maria Roes, E., Raijmakers, M. T., Steegers, E. A., Peters, W. H., & Smits, P. (2004). *N*-acetylcysteine restores nitric oxide-mediated effects in the fetoplacental circulation of preeclamptic patients. *Am J Obstet Gynecol 191*, 328–333.

Blair, I. A. (2001). Lipid hydroperoxide-mediated DNA damage. *Exp Gerontol 36*, 1473–1481.

Boger, R. H., Bode-Boger, S. M., Szuba, A., Tsao, P. S., Chan, J. R., Tangphao, O., et al. (1998). Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation 98*, 1842–1847.

Brigelius-Flohe, R., Kelly, F. J., Salonen, J. T., Neuzil, J., Zingg, J. M., & Azzi, A. (2002). The European perspective on vitamin E: current knowledge and future research. *Am J Clin Nutr 76*, 703–716.

Burton, G. J., & Jauniaux, E. (2004). Placental oxidative stress: from miscarriage to preeclampsia. *J Soc Gynecol Investig 11*, 342–352.

Burton, G. J., Jauniaux, E., & Watson, A. L. (1999). Maternal arterial connections to the placental intervillous space during the first trimester of human pregnancy: the Boyd collection revisited. *Am J Obstet Gynecol 181*, 718–724.

Cachia, O., Benna, J. E., Pedruzzi, E., Descomps, B., Gougerot-Pocidalo, M. A., & Leger, C. L. (1998). Alpha-tocopherol inhibits the respiratory burst in human monocytes. Attenuation of p47 (phox) membrane translocation and phosphorylation. *J Biol Chem 273*, 32801–32805.

Clausen, T., Djurovic, S., & Henriksen, T. (2001). Dyslipidemia in early second trimester is mainly a feature of women with early onset preeclampsia. *Br J Obstet Gynecol 108*, 1081–1087.

Coffey, C. G. (2003). Cellular bases for the lipid-related aspects of preeclampsia. *Med Hypotheses 60*, 716–719.

Cooke, C. L., & Davidge, S. T. (2002). Peroxynitrite increases iNOS through NF-κB and decreases prostacyclin synthase in endothelial cells. *Am J Physiol Cell Physiol 282*, C395–C402.

Chambers, C. J., Ueland, P. M., Wright, M., Dore, C. J., Refsum, H., & Kooner, J. S. (2001). Investigation of relationship between reduced, oxidized, and protein-bound homocysteine and vascular endothelial function in healthy human subjects. *Circ Res 89*, 187–192.

Chan, A. C. (1993). Partners in defense, vitamin E and vitamin C. *Can J Physiol Pharmacol 71*, 725–731.

Chappell, L. C., Seed, P. T., Briley, A. L., Kelly, F. J., Lee, R., Hunt, B. J., et al. (1999). Effect of antioxidants on the occurrence of pre-eclampsia in women at increased risk: a randomised trial. *Lancet 354*, 810–816.

Chappell, L. C., Seed, P. T., Briley, A., Kelly, F. J., Hunt, B. J., Charnock-Jones, D. S., et al. (2002a). A longitudinal study of biochemical variables in women at risk of preeclampsia. *Am J Obstet Gynecol 187*, 127–136.

Chappell, L. C., Seed, P. T., Kelly, F. J., Briley, A., Hunt, B. J., Charnock-Jones, D. S., et al. (2002b). Vitamin C and E supplementation in women at risk of preeclampsia is associated with changes in indices of oxidative stress and placental function. *Am J Obstet Gynecol 187*, 777–784.

Chaudiere, J., & Ferrari-Iliou, R. (1999). Intracellular antioxidants: from chemical to biochemical mechanisms. *Food Chem Toxicol 37*, 949–962.

Chen, X., Touyz, R. M., Park, J. B., & Schiffrin, E. L. (2001). Antioxidant effects of vitamins C and E are associated with altered activation of vascular NADPH oxidase and superoxide dismutase in stroke-prone SHR. *Hypertension 38*(3 Pt 2), 606–611.

Choi, J. W., Im, M. W., & Pai, S. H. (2002). Nitric oxide production increases during normal pregnancy and decreases in preeclampsia. *Ann Clin Lab Sci 32*, 257–263.

Conrad, K. P., Kerchner, L. J., & Mosher, M. D. (1999). Plasma and 24-h NO(x) and cGMP during normal pregnancy and preeclampsia in women on a reduced NO(x) diet. *Am J Physiol 277*(1 Pt 2), F48–F57.

Cracowski, J. L., Devillier, P., Durand, T., Stanke-Labesque, F., & Bessard, G. (2001). Vascular biology of the isoprostanes. *J Vasc Res 38*, 93–103.

da Costa, B. P., Steibel, J. P., Antonello, I. C., Guimaraes, J. A, & Poli de Figueiredo, C. E. (2004). L-Arginine erythrocyte transport in normal pregnant and preeclamptic women. *Am J Obstet Gynecol 190*, 468–471.

Dai, Z., Zhu, H. Q., Jiang, D. J., Jiang, J. L., Deng, H. W., & Li, Y. J. (2004). 17Beta-estradiol preserves endothelial function by reduction of the endogenous nitric oxide synthase inhibitor level. *Int J Cardiol 96*, 223–227.

D'Anna, R., Baviera, G., Corrado, F., Ientile, R., Granese, D., & Stella, N. C. (2004). Plasma homocysteine in early and late pregnancies complicated with preeclampsia and isolated intrauterine growth restriction. *Acta Obstet Gynecol Scand 83*, 155–158.

Davidge, S. T., Stranko, C. P., & Roberts, J. M. (1996). Urine but not plasma nitric oxide metabolites are decreased in women with preeclampsia. *Am J Obstet Gynecol 174*, 1008–1113.

Davison, J. M., Homuth, V., Jeyabalan, A., Conrad, K. P., Karumanchi, S. A., & Quaggin, S., et al. (2004). New aspects in the pathophysiology of preeclampsia. *J Am Soc Nephrol 15*, 2440–2448.

Dechend, R., Viedt, C., Muller, D. N., Ugele, B., Brandes, R. P., Wallukat, G., et al. (2003). AT1 receptor agonistic antibodies from preeclamptic patients stimulate NADPH oxidase. *Circulation 107*, 1632–1639.

Delles, C., Schneider, M. P., John, S., Gekle, M., & Schmieder, R. E. (2002). Angiotensin converting enzyme inhibition and angiotensin II AT1-receptor blockade reduce the levels of asymmetrical N(G), N(G)-dimethylarginine in human essential hypertension. *Am J Hypertens 15*(7 Pt 1), 590–593.

Diejomaoh, F. M., Omu, A. E., Al-Busiri, N., Taher, S., Al-Othman, S., Fatinikun, T., et al. (2004). Nitric oxide production is not altered in preeclampsia. *Arch Gynecol Obstet 269*, 237–243.

Dikalov, S. I., Dikalova, A. E., & Mason, R. P. (2002). Noninvasive diagnostic tool for inflammation-induced oxidative stress using electron spin resonance spectroscopy and an extracellular cyclic hydroxylamine. *Arch Biochem Biophys 402*, 218–226.

Di Simone, N., Maggiano, N., Caliandro, D., Riccardi, P., Evangelista, A., Carducci, B., et al. (2003). Homocysteine induces trophoblast cell death with apoptotic features. *Biol Reprod 69*, 1129–1134.

Ding, Z. Q., Rowe, J., Ng, B., Sinosich, M. J., & Gallery, E. D. (2002). Modulation of prostacyclin and thromboxane secretion by cytotrophoblasts from normal and pre-eclamptic human pregnancies. *Placenta 23*, 594–599.

Dotsch, J., Hogen, N., Nyul, Z., Hanze, J., Knerr, I., Kirschbaum, M., et al. (2001). Increase of endothelial nitric oxide synthase and endothelin-1 mRNA expression in human placenta during gestation. *Eur J Obstet Gynecol Reprod Biol 97*, 163–167.

El-Salahy, E. M., Ahmed, M. I., El-Gharieb, A., & Tawfik, H. (2001). New scope in angiogenesis: role of vascular endothelial growth factors (VEGF), NO, lipid peroxidation, and vitamin E in the pathophysiology of pre-eclampsia among Egyptian females. *Clin Biochem 34*, 323–329.

Endemann, D. H., & Schiffrin, E. L. (2004). Endothelial dysfunction. *J Am Soc Nephrol 15*, 1983–1992.

Endresen, M. J., Tosti, E., Heimli, H., Lorentzen, B., & Henriksen, T. (1994). Effects of free fatty acids found increased in women who develop pre-eclampsia on the ability of endothelial cells to produce prostacyclin, cGMP and inhibit platelet aggregation. *Scand J Clin Lab Invest 54*, 549–557.

Enquobahrie, D. A., Williams, M. A., Butler, C. L., Frederick, I. O., Miller, R. S., & Luthy, D. A. (2004). Maternal plasma lipid concentrations in early pregnancy and risk of preeclampsia. *Am J Hypertens 17*, 574–581.

Fukunaga, M., Makita, N., Roberts, L. J., Morrow, J. D., Takahashi, K., & Badr, K. F. (1993). Evidence for the existence of F2-isoprostane receptors on rat vascular smooth muscle cells. *Am J Physiol 264*(6 Pt 1), C1619–C1624.

Fukunaga, M., Yura, T., & Badr, K. F. (1995). Stimulatory effect of 8-epi-PGF_{2alpha}, an F2-isoprostane, on endothelin-1 release. *J Cardiovasc Pharmacol 26*, S51–S52.

Gerber, H. P., Condorelli, F., Park, J., & Ferrara, N. (1997). Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J Biol Chem 272*, 23659–23667.

Gratacos, E. (2000). Lipid-mediated endothelial dysfunction: a common factor to preeclampsia and chronic vascular disease. *Eur J Obstet Gynecol Reprod Biol 92*, 63–66.

Gratacos, E., Casals, E., Sanllehy, C., Cararach, V., Alonso, P. L., & Fortuny, A. (1996). Variation in lipid levels during pregnancy in women with different types of hypertension. *Acta Obstet Gynecol Scand 75*, 896–901.

Gratacos, E., Casals, E., Gomez, O., Llurba, E., Mercader, I., Cararach, V., et al. (2003). Increased susceptibility to low density lipoprotein oxidation in women with a history of pre-eclampsia. *Br J Obstet Gynaecol 110*, 400–404.

Hague, W. M. (2003). Homocysteine and pregnancy. *Best Pract Res Clin Obstet Gynaecol 17*, 459–469.

Hassoun, P. M., Yu, F. S., Shedd, A. L., Zulueta, J. J., Thannickal, V. J., Lanzillo, J. J., et al. (1994). Regulation of endothelial cell xanthine dehydrogenase xanthine oxidase gene expression by oxygen tension. *Am J Physiol 266*(2 Pt 1), L163–L171.

Holden, D. P., Fickling, S. A., Whitley, G. S., & Nussey, S. S. (1998). Plasma concentrations of asymmetric dimethylarginine, a natural inhibitor of nitric oxide synthase, in normal pregnancy and preeclampsia. *Am J Obstet Gynecol 178*, 551–556.

Holthe, M. R., Staff, A. C., Berge, L. N., & Lyberg, T. (2004). Leukocyte adhesion molecules and reactive oxygen species in preeclampsia. *Obstet Gynecol 103*(5 Pt 1), 913–922.

Horwitt, M. K., Harvey, C. C., Dahm Jr., C. H., & Searcy, M. T. (1972). Relationship between tocopherol and serum lipid levels for determination of nutritional adequacy. *Ann N Y Acad Sci 203*, 223–236.

Hubel, C. A. (1999). Oxidative stress in the pathogenesis of preeclampsia. *Proc Soc Exp Biol Med 222*, 222–235.

Hubel, C. A., McLaughlin, M. K., Evans, R. W., Hauth, B. A., Sims, C. J., & Roberts, J. M. (1996). Fasting serum triglycerides, free fatty acids, and malondialdehyde are increased in preeclampsia, are positively correlated, and decrease within 48 hours post partum. *Am J Obstet Gynecol 174*, 975–982.

Hubel, C. A., Kagan, V. E., Kisin, E. R., McLaughlin, M. K., & Roberts, J. M. (1997). Increased ascorbate radical formation and ascorbate. *Free Radic Biol Med 23*, 597–609.

Hubel, C. A., Lyall, F., Weissfeld, L., Gandley, R. E., & Roberts, J. M. (1998). Small low-density lipoproteins and vascular cell adhesion molecule-1 are increased in association with hyperlipidemia in preeclampsia. *Metabolism 47*, 1281–1288.

Huie, R. E., & Padmaja, S. (1993). The reaction of NO with superoxide. *Free Radic Res Commun 18*, 195–199.

Hung, T. H., Skepper, J. N., & Burton, G. J. (2001). In vitro ischemia–reperfusion injury in term human placenta as a model for oxidative stress in pathological pregnancies. *Am J Pathol 159*, 1031–1043.

Hung, T. H., Skeeper, J. N., Charnock-Jones, D. S., & Burton, G. J. (2002). Hypoxia-reoxygenation: a potent inducer of apoptotic changes in the human placenta and possible etiological factor in preeclampsia. *Circ Res 90*, 1274–1281.

Ischiropoulos, H., Zhu, I., Chen, J., Tsai, M., Martin, J. C., Smith, C. D., et al. (1992). Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Arch Biochem Biophys 298*, 431–437.

Jauniaux, E., Watson, A. L., Hempstock, J., Bao, Y. P., Skepper, J. N., & Burton, G. J. (2000). Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. *Am J Pathol 157*, 2111–2122.

Jauniaux, E., Cindrova-Davies, T., Johns, J., Dunster, C., Hempstock, J., Kelly, F. J., & Burton, G. J. (2004). Distribution and transfer pathways of antioxidant molecules inside the first trimester human gestational sac. *J Clin Endocrinol Metab 89*, 1452–1458.

Karumanchi, S. A., & Bdolah, Y. (2004). Hypoxia and sFlt-1 in preeclampsia: the "chicken-and-egg" question. *Endocrinology 145*, 4835–4837.

Kauma, S., Takacs, P., Scordalakes, C., Walsh, S., Green, K., & Peng, T. (2002). Increased endothelial monocyte chemoattractant protein-1 and interleukin-8 in preeclampsia. *Obstet Gynecol 100*, 706–714.

Kharb, S. (2000). Vitamin E and C in preeclampsia. *Eur J Obstet Reprod Biol 93*, 37–39.

Klein, T., Reutter, F., Schweer, H., Seyberth, H. W., & Nusing, R. M. (1997). Generation of the 8-*epi*-prostaglandin F2alpha in vitro and in vivo via the cyclooxygenases. *J Pharmacol Exp Ther 282*, 1658–1665.

Kubes, P., Suzuki, M., & Granger, D. N. (1991). Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci U S A 88*, 4651–4655.

Kumar, C. A., & Das, U. N. (2000). Lipid peroxides, anti-oxidants and nitric oxide in patients with pre-eclampsia and essential hypertension. *Med Sci Monit 6*, 901–907.

Lee, J. M., & Johnson, J. A. (2004). An important role of Nrf2–ARE pathway in the cellular defense mechanism. *J Biochem Mol Biol 37*, 139–143.

Lee, V. M., Quinn, P. A., Jennings, S. C., & Ng, L. L. (2003a). Neutrophil activation and production of reactive oxygen species in preeclampsia. *J Hypertens 21*, 395–402.

Lee, V. M., Quinn, P. A., Jennings, S. C., & Ng, L. L. (2003b). NADPH oxidase activity in preeclampsia with immortalized lymphoblasts used as models. *Hypertension 41*, 925–931.

Lee, B. E., Hong, Y. C., Lee, K. H., Kim, Y. J., Kim, W. K., Chang, N. S., et al. (2004). Influence of maternal serum levels of vitamins C and E during the second trimester on birth weight and length. *Eur J Clin Nutr 58*, 1365–1371.

Leger, C. L., Dumontier, C., Fouret, G., Boulot, P., & Descomps, B. (1998). A short-term supplementation of pregnant women before delivery does not improve significantly the vitamin E status of neonates-low efficiency of the vitamin E placental transfer. *Int J Vitam Nutr Res 68*, 293–299.

Levine, R. J., Maynard, S. E., Qian, C., Lim, K. H., England, L. J., Yu, K. F., et al. (2004). Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med 350*, 672–683.

Liochev, S. I., & Fridovich, I. (1997). How does superoxide dismutase protect against tumor necrosis factor: a hypothesis informed by effect of superoxide on "free" iron. *Free Radic Biol Med 23*, 668–671.

Liu, J., Yeo, H. C., Doniger, S. J., & Ames, B. N. (1997). Assay of aldehydes from lipid peroxidation: gas chromatography-mass spectrometry compared to thiobarbituric acid. *Anal Biochem 245*, 161–166.

Liu, T. Z., Stern, A., & Morrow, J. D. (1998). The isoprostanes: unique bioactive products of lipid peroxidation. An overview. *J Biomed Sci 5*, 415–420.

Llurba, E., Gratacos, E., Martin-Gallan, P., Cabero, L., & Dominguez, C. (2004). A comprehensive study of oxidative stress and antioxidant status in preeclampsia and normal pregnancy. *Free Radic Biol Med 37*, 557–570.

Lorentzen, B., & Henriksen, T. (1998). Plasma lipids and vascular dysfunction in preeclampsia. *Semin Reprod Endocrinol 16*, 33–39.

Luscher, T. F., & Barton, M. (1997). Biology of the endothelium. *Clin Cardiol 20*(11 Suppl 2), II3–II10.

Luttun, A., & Carmeliet, P. (2003). Soluble VEGF receptor Flt1: the elusive preeclampsia factor discovered? *J Clin Invest 111*, 600–602.

Maas, R., Boger, R. H., Schwedhelm, E., Casas, J. P., Lopez-Jaramillo, P., Serrano, N., et al. (2004). Plasma concentrations of asymmetric dimethylarginine (ADMA) in Colombian women with pre-eclampsia. *J Am Med Assoc 291*, 823–824.

Madazli, R., Benian, A., Gumustas, K., Uzun, H., Ocak, V., & Aksu, F. (1999). Lipid peroxidation and antioxidant in preeclampsia. *Eur J Obstet Gynecol Reprod Biol 85*, 205–208.

Madazli, R., Benian, A., Aydin, S., Uzun, H., & Tolun, N. (2002). The plasma and placental levels of malondialdehyde, glutathione and superoxide dismutase in pre-eclampsia. *J Obstet Gynaecol 22*, 477–480.

Manes, C. (2001). Human placental NAD(P)H oxidase: solubilization and properties. *Placenta 22*, 58–63.

Many, A., Hubel, C. A., Fisher, S. J., Roberts, J. M., & Zhou, Y. (2000). Invasive cytotrophoblasts manifest evidence of oxidative stress in preeclampsia. *Am J Pathol 156*, 321–331.

Martinez-Orgado, J., Gonzalez, R., Alonso, M. J., & Salaices, M. (2004). Impairment of fetal endothelium-dependent relaxation in a rat model of preeclampsia by chronic nitric oxide synthase inhibition. *J Soc Gynecol Investig 11*, 82–88.

Matsubara, S., & Sato, I. (2001). Enzyme histochemically detectable NAD(P)H oxidase in human placental trophoblast: normal, preeclamptic, and fetal growth restriction-complicated pregnancy. *Histochem Cell Biol 116*, 1–7.

Matsubara, S., Takayama, T., Iwasaki, R., Komatsu, N., Matsubara, D., Takizawa, T., et al. (2001). Enzyme-cytochemically detectable glucose-6-phosphate dehydrogenase in human villous macrophages (Hofbauer cells). *Placenta 22*, 882–885.

Maulik, N., & Das, D. K. (2002). Redox signaling in vascular angiogenesis. *Free Radic Biol Med 33*, 1047–1060.

Maynard, S. E., Min, J. Y., Merchan, J., Lim, K. H., Li, J., Mondal, S., et al. (2003). Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest 111*, 649–658.

McCracken, S. A., Drury, C. L., Lee, H. S., & Morris, J. M. (2003). Pregnancy is associated with suppression of the nuclear factor kappaB/IkappaB activation pathway in peripheral blood mononuclear cells. *J Reprod Immunol 58*, 27–47.

McGiff, J. C., & Quilley, J. (2001). 20-Hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids and blood pressure. *Curr Opin Nephrol Hypertens 10*, 231–237.

McKinney, E. T., Shouri, R., Hunt, R. S., Ahokas, R. A., & Sibai, B. M. (2000). Plasma, urinary, and salivary 8-*epi*-prostaglandin f2alpha levels in normotensive and preeclamptic pregnancies. *Am J Obstet Gynecol 183*, 874–877.

Merviel, P., Carbillon, L., Challier, J. C., Rabreau, M., Beaufils, M., & Uzan, S. (2004). Pathophysiology of preeclampsia: links with implantation disorders. *Eur J Obstet Gynecol Reprod Biol 115*, 134–147.

Mikhail, M. S., Anyaegbunam, A., Garfinkel, D., Palan, P. R., Basu, J., & Romney, S. L. (1994). Preeclampsia and antioxidant nutrients: decreased plasma levels of reduced ascorbic acid, alpha-tocopherol,

and beta-carotene in women with preeclampsia. *Am J Obstet Gynecol 17*, 150–157.

Milczarek, R., Klimek, J., & Zelewski, L. (2000). The effects of ascorbate and alpha-tocopherol on the NADPH-dependent lipid peroxidation in human placentalmitochondria. *Mol Cell Biochem 210*, 65–73.

Milstien, S., & Katusic, Z. (1999). Oxidation of tetrahydrobiopterin by peroxynitrite: implications for vascular endothelial function. *Biochem Biophys Res Commun 263*, 681–684.

Mohindra, A., Kabi, B. C., Kaul, N., & Trivedi, S. S. (2002). Vitamin E and carotene status in pre-eclamptic pregnant women from India. *Panminerva Med 44*, 261–264.

Moran, J. P., Cohen, L., Greene, J. M., Xu, G., Feldman, E. B., Hames, C. G., et al. (1993). Plasma ascorbic acid concentrations relate inversely to blood pressure in human subjects. *Am J Clin Nutr 57*, 213–217.

Moretti, M., Phillips, M., Abouzeid, A., Cataneo, R. N., & Greenberg, J. (2004). Increased breath markers of oxidative stress in normal pregnancy and in preeclampsia. *Am J Obstet Gynecol 190*, 1184–1190.

Morrow, J. D. (2000). The isoprostanes: their quantification as an index of oxidant stress status in vivo. *Drug Metab Rev 32*, 377–385.

Mutlu-Turkoglu, U., Ademoglu, E., Ibrahimoglu, L., Aykac-Toker, G., & Uysal, M. (1998). Imbalance between lipid peroxidation and antioxidant status in preeaclampsia. *Gynecol Obstet Invest 46*, 37–40.

Myatt, L., & Cui, X. (2004). Oxidative stress in the placenta. *Histochem Cell Biol 122*, 369–382.

Myatt, L. M., Rosenfield, R. B., Eis, A. L. M., Brockman, D. E., Greer, I., & Lyall, F. (1996). Nitrotyrosine residues in placenta: evidence of peroxynitrite formation and action. *Hypertension 28*, 488–498.

Myatt, L., Eis, A. L., Brockman, D. E., Kossenjans, W., Greer, I., & Lyall, F. (1997). Inducible (type II) nitric oxide synthase in human placental villous tissue of normotensive, pre-eclamptic and intrauterine growth-restricted pregnancies. *Placenta 18*, 261–268.

Nagamatsu, T., Fujii, T., Kusumi, M., Zou, L., Yamashita, T., Osuga, Y., et al. (2004). Cytotrophoblasts up-regulate soluble fms-like tyrosine kinase-1 expression under reduced oxygen: an implication for the placental vascular development and the pathophysiology of preeclampsia. *Endocrinology 145*, 4838–4845.

Napolitano, M., Miceli, F., Calce, A., Vacca, A., Gulino, A., Apa, R., et al. (2000). Expression and relationship between endothelin-1 messenger ribonucleic acid (mRNA) and inducible/endothelial nitric oxide synthase mRNA isoforms from normal and preeclamptic placentas. *J Clin Endocrinol Metab 85*, 2318–2323.

Newaz, M. A., Nawal, N. N., Rohaizan, C. H., Muslim, N., & Gapor, A. (1999). Alpha-tocopherol increased nitric oxide synthase activity in blood vessels of spontaneously hypertensive rats. *Am J Hypertens 12*(8 Pt 1), 839–844.

Noris, M., Todeschini, M., Cassis, P., Pasta, F., Cappellini, A., Bonazzola, S., et al. (2004). L-Arginine depletion in preeclampsia orients nitric oxide synthase toward oxidant species. *Hypertension 43*, 614–622.

Noto, R., Neri, S., Noto, Z., Cilio, D., Abate, G., Noto, P., et al. (2003). Hyperhomocysteinemia in preeclampsia is associated to higher risk pressure profiles. *Eur Rev Med Pharmacol Sci 7*, 81–87.

Nobunaga, T., Tokugawa, Y., Hashimoto, K., Kimura, T., Matsuzaki, N., Nitta, Y., et al. (1996). Plasma nitric oxide levels in pregnant patients with preeclampsia and essential hypertension. *Gynecol Obstet Invest 41*, 189–193.

Nunes, G. L., Robinson, K., Kalynych, A., King 3rd, S. B., Sgoutas, D. S., & Berk, B. C. (1997). Vitamins C and E inhibit O2-production in the pig coronary artery. *Circulation 96*, 3593–3601.

Okatani, Y., Watanabe, K., & Sagara, Y. (1997). Effect of nitric oxide, prostacyclin, and thromboxane on the vasospastic action of hydrogen peroxide on human umbilical artery. *Acta Osbtet Gynecol Scand 76*, 515–520.

Orange, S. J., Painter, D., Horvath, J., Yu, B., Trent, R., & Hennessy, A. (2003). Placental endothelial nitric oxide synthase localization and expression in normal human pregnancy and pre-eclampsia. *Clin Exp Pharmacol Physiol 30*, 376–381.

Palan, P. R., Mikhail, M. S., & Romney, S. L. (2001). Placental and serum levels of carotenoids in preeclampsia. *Obstet Gynecol 98*, 459–462.

Palan, P. R., Shaban, D. W., Martino, T., & Mikhail, M. S. (2004). Lipid-soluble antioxidants and pregnancy: maternal serum levels of coenzyme Q10, alpha-tocopherol and gamma-tocopherol in preeclampsia and normal pregnancy. *Gynecol Obstet Invest 58*, 8–13.

Panburana, P., Phuapradit, W., & Puchaiwatananon, O. (2000). Antioxidants nutrients and lipid peroxide levels in Thai preeclamptic pregnant women. *J Obstet Gynaecol Res 26*, 377–381.

Patrick, T. E., Hubel, C. A., & Roberts, J. M. (2004). Evidence of increased oxidative stress, unexplained by lipid changes, is present in nulliparous black women from early gestation. *Hypertens Pregnancy 23*, 91–100.

Pavan, L., Tsatsaris, V., Hermouet, A., Therond, P., Evain-Brion, D., & Fournier, T. (2004). Oxidized low-density lipoproteins inhibit trophoblastic cell invasion. *J Clin Endocrinol Metab 89*, 1969–1972.

Pettersson, A., Hedner, T., & Milsom, I. (1998). Increased circulating concentrations of asymmetric dimethyl arginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, in preeclampsia. *Acta Obstet Gynecol Scand 77*, 808–813.

Plessinger, M. A., Woods Jr., J. R., & Miller, R. K. (2000). Pretreatment of human amnion-chorion with vitamins C and E prevents hypochlorous acid-induced damage. *Am J Obstet Gynecol 183*, 979–985.

Poston, L., & Raijmakers, M. T. (2004). Trophoblast oxidative stress, antioxidants and pregnancy outcome (a review). *Placenta 25*(Suppl A), S72–S78.

Powers, R. W., Majors, A. K., Kerchner, L. J., & Conrad, K. P. (2004). Renal handling of homocysteine during normal pregnancy and preeclampsia. *J Soc Gynecol Investig 11*, 45–50.

Pratico, D., Lawson, J. A., Rokach, J., & Fitzgerald, G. A. (2001). The isoprostanes in biology and medicine. *Trends Endocrinol Metab 12*, 243–247.

Pressman, E. K., Cavanaugh, J. L., Mingione, M., Norkus, E. P., & Woods, J. R. (2003). Effects of maternal antioxidant supplementation on maternal and fetal antioxidant levels: a randomized, double-blind study. *Am J Obstet Gynecol 189*, 1720–1725.

Qanungo, S., & Mukherjea, M. (2000). Ontogenic profile of some antioxidants and lipid peroxidation in human placental and fetal tissues. *Mol Cell Biochem 215*, 11–19.

Radomski, M. W., Palmer, R. M., & Moncada, S. (1987). The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. *Br J Pharmacol 92*, 639–646.

Raijmakers, M. T., Dechend, R., & Poston, L. (2004a). Oxidative stress and preeclampsia: rationale for antioxidant clinical trials. *Hypertension 44*, 374–380.

Raijmakers, M. T., Roes, E. M., Zusterzeel, P. L., Steegers, E. A., & Peters, W. H. (2004b). Thiol status and antioxidant capacity in women with a history of severe pre-eclampsia. *Br J Obstet Gynaecol 111*, 207–212.

Regan, C. L., Levine, R. J., Baird, D. D., Ewell, M. G., Martz, K. L., Sibai, B. M., et al. (2001). No evidence for lipid peroxidation in severe preeclampsia. *Am J Obstet Gynecol 185*, 572–578.

Roberts, J. M. (1998). Endothelial dysfunction in preeclampsia. *Semin Reprod Endocrinol 16*, 5–15.

Roberts, J. M., & Cooper, D. W. (2001). Pathogenesis and genetics of pre-eclampsia. *Lancet 357*, 53–56.

Roberts, J. M., & Hubel, C. A. (1999). Is oxidative stress the link in the two-stage model of pre-eclampsia? *Lancet 354*, 788–789.

Rodrigo, R., Passalacqua, W., Araya, J., Orellana, M., & Rivera, G. (2003). Homocysteine and essential hypertension. *J Clin Pharmacol 43*, 1299–1306.

Sagol, S., Ozkinay, E., & Ozsener, S. (1999). Impaired antioxidant activity in women with pre-eclampsia. *Int J Gynaecol Obstet 64*, 121–127.

Salonen, J. T., Nyyssonen, K., Salonen, R., Lakka, H. M., Kaikkonen, J., Porkkala-Sarataho, E., et al. (2000). Antioxidant Supplementation in Atherosclerosis Prevention (ASAP) study: a randomized trial of the

effect of vitamins E and C on 3-year progression of carotid atherosclerosis. *J Intern Med 248*, 377–386.

Sattar, N., Bendomir, A., Berry, C., Shepherd, J., Greer, I. A., & Packard, C. J. (1997). Lipoprotein subfraction concentrations in preeclampsia: pathogenic parallels to atherosclerosis. *Obstet Gynecol 89*, 403–408.

Savvidou, M. D., Hingorani, A. D., Tsikas, D., Frolich, J. C., Vallance, P., & Nicolaides, K. H. (2003). Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. *Lancet 361*, 1511–1517.

Schiff, E., Friedman, S. A., Stampfer, M., Kao, L., Barrett, P. H., & Sibai, B. M. (1996). Dietary consumption and plasma concentrations of vitamin E in pregnancies complicated by preeclampsia. *Am J Obstet Gynecol 175*(4 Pt 1), 1024–1028.

Schlembach, D., Scalera, F., Fischer, T., Marx, S. G., Beinder, E., & Garfield, R. E. (2003). Neurokinin B peptide serum levels are higher in normotensive pregnant women than in preeclamptic pregnant women. *Am J Obstet Gynecol 189*, 1418–1422.

Scholz, H., Yndestad, A., Damas, J. K., Waehre, T., Tonstad, S., Aukrust, P., et al. (2003). 8-Isoprostane increases expression of interleukin-8 in human macrophages through activation of mitogen-activated protein kinases. *Cardiovasc Res 59*, 945–954.

Seligman, S. P., Buyon, J. P., Clancy, R. M., Young, B. K., & Abramson, S. B. (1994). The role of nitric oxide in the pathogenesis of preeclampsia. *Am J Obstet Gynecol 171*, 944–948.

Serdar, Z., Gur, E., Develioglu, O., Colakogullari, M., & Dirican, M. (2002). Placental and decidual lipid peroxidation and antioxidant defenses in preeclampsia. Lipid peroxidation in preeclampsia. *Pathophysiology 9*, 21.

Serdar, Z., Gur, E., Colakoethullary, M., Develioethlu, O., & Sarandol, E. (2003). Lipid and protein oxidation and antioxidant function in women with mild and severe preeclampsia. *Arch Gynecol Obstet 268*, 19–25.

Sies, H. (1991). Role of reactive oxygen species in biological processes. *Klin Wochenschr 69*, 965–968.

Sikkema, J. M., van Rijn, B. B., Franx, A., Bruinse, H. W., de Roos, R., Stroes, R., et al. (2001). Placental superoxide is increased in pre-eclampsia. *Placenta 22*, 304–308.

Silver, R. K., Kupferminc, M. J., Russell, T. L., Adler, L., Mullen, T. A., & Caplan, M. S. (1996). Evaluation of nitric oxide as a mediator of severe preeclampsia. *Am J Obstet Gynecol 175*(4 Pt 1), 1013–1017.

Slatter, D. A., Bolton, C. H., & Bailey, A. J. (2000). The importance of lipid-derived malondialdehyde in diabetes mellitus. *Diabetologia 43*, 550–557.

Smárason, A. K., Allman, K. G., Young, D., & Redman, C. W. (1997). Elevated levels of serum nitrate, a stable end product of nitric oxide, in women with pre-eclampsia. *Br J Obstet Gynaecol 104*, 538–543.

Staff, A. C., Halvorsen, B., Ranheim, T., & Henriksen, T. (1999). Elevated level of free 8-iso-prostaglandin F2alpha in the decidua basalis of women with preeclampsia. *Am J Obstet Gynecol 181*(5 Pt 1), 1211–1215.

Stuhlinger, M. C., Oka, R. K., Graf, E. E., Schmolzer, I., Upson, B. M., Kapoor, O., et al. (2003). Endothelial dysfunction induced by hyperhomocyst(e)inemia: role of asymmetric dimethylarginine. *Circulation 108*, 933–938.

Sugimoto, H., Hamano, Y., Charytan, D., Cosgrove, D., Kieran, M., Sudhakar, A., et al. (2003). Neutralization of circulating vascular endothelial growth factor (VEGF) by anti-VEGF antibodies and soluble VEGF receptor 1 (sFlt-1) induces proteinuria. *J Biol Chem 278*, 12605–12608.

Surdacki, A., Nowicki, M., Sandmann, J., Tsikas, D., Boeger, R. H., Bode-Boeger, S. M., et al. (1999). Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. *J Cardiovasc Pharmacol 33*, 652–658.

Sydow, K., Schwedhelm, E., Arakawa, N., Bode-Boger, S. M., Tsikas, D., Hornig, B., et al. (2003). ADMA and oxidative stress are responsible for endothelial dysfunction in hyperhomocyst(e)inemia: effects of L-arginine and B vitamins. *Cardiovasc Res 57*, 244–252.

Szabo, I., Vizer, M., & Ertl, T. (2003). Fetal betamethasone treatment and neonatal outcome in preeclampsia and intrauterine growth restriction. *Am J Obstet Gynecol 189*, 1812–1813.

Taddei, S., Virdis, A., Ghiadoni, L., & Salvetti, A. (1998). The role of endothelium in human hypertension. *Curr Opin Nephrol Hypertens 7*, 203–209.

Takacs, P., Kauma, S. W., Sholley, M. M., Walsh, S. W., Dinsmoor, M. J., & Green, K. (2001). Increased circulating lipid peroxides in severe preeclampsia activate NF-kappaB and upregulate ICAM-1 in vascular endothelial cells. *FASEB J 15*, 279–281.

Takacs, P., Green, K. L., Nikaeo, A., & Kauma, S. W. (2003). Increased vascular endothelial cell production of interleukin-6 in severe pre-eclampsia. *Am J Obstet Gynecol 188*, 740–744.

Takagi, Y., Nikaido, T., Toki, T., Kita, N., Kanai, M., Ashida, T., et al. (2004). Levels of oxidative stress and redox-related molecules in the placenta in preeclampsia and fetal growth restriction. *Virchows Arch 444*, 49–55.

Talalay, P., Dinkova-Kostova, A. T., & Holtzclaw, W. D. (2003). Importance of phase 2 gene regulation in protection against electrophile and reactive oxygen toxicity and carcinogenesis. *Adv Enzyme Regul 43*, 121–134.

Tamura, T., Olin, K. L., Goldenberg, R. L., Johnston, K. E., Dubard, M. B., & Keen, C. L. (2001). Plasma extracellular superoxide dismutase activity in healthy pregnant women is not influenced by zinc supplementation. *Biol Trace Elem Res 80*, 107–113.

Touyz, R. M., & Schiffrin, E. L. (2004). Reactive oxygen species in vascular biology: implications in hypertension. *Histochem Cell Biol 122*, 339–352.

Tranquilli, A. L., Bezzeccheri, V., Giannubilo, S. R., Scagnoli, C., Mazzanti, L., & Garzetti, G. G. (2004). Amniotic vascular endothelial growth factor (VEGF) and nitric oxide (NO) in women with subsequent preeclampsia. *Eur J Obstet Gynecol Reprod Biol 113*, 17–20.

Tribble, D. L., Thiel, P. M., van den Berg, J. J., & Krauss, R. M. (1995). Differing alpha-tocopherol oxidative lability and ascorbic acid sparing effects in buoyant and dense LDL. *Arterioscler Thromb Vasc Biol 15*, 2025–2031.

Tug, N., Celik, H., Cikim, G., Ozcelik, O., & Ayar, A. (2003). The correlation between plasma homocysteine and malondialdehyde levels in preeclampsia. *Neuro Endocrinol Lett 24*, 445–448.

Ubbink, J. B., van der Merwe, A., Vermaak, W. J., & Delport, R. (1993). Hyperhomocysteinemia and the response to vitamin supplementation. *Clin Invest 71*, 993–998.

Ülker, S., McKeown, P. P., & Bayraktutan, U. (2003). Vitamins reverse endothelial dysfunction through regulation of eNOS and NAD(P)H oxidase activities. *Hypertension 41*, 534–539.

Uotila, J., Tuimala, R., Aarnio, T., Pyykko, K., & Ahotupa, M. (1991). Lipid peroxidation products, selenium-dependent glutathione peroxidase and vitamin E in normal pregnancy. *Eur J Obstet Gynecol Reprod Biol 42*, 95–100.

Uotila, J. T., Tuimala, R. J., Aarnio, T. M., Pyykko, K. A., & Ahotupa, M. O. (1993). Findings on lipid peroxidation and antioxidant function in hypertensive complications of pregnancy. *Br J Obstet Gynaecol 100*, 270–276.

Ushio-Fukai, M., Tang, Y., Fukai, T., Dikalov, S. I., Ma, Y., Fujimoto, M., et al. (2002). Novel role of gp91(phox)-containing NAD(P)H oxidase in vascular endothelial growth factor-induced signaling and angiogenesis. *Circ Res 91*, 1160–1167.

Vadachkoria, S., Sanchez, S. E., Qiu, C., Muy-Rivera, M., Malinow, M. R., & Williams, M. A. (2004). Hyperhomocyst(e)inemia and elevated soluble vascular cell adhesion molecule-1 concentrations are associated with an increased risk of preeclampsia. *Gynecol Obstet Invest 58*, 133–139.

Var, A., Kuscu, N. K., Koyuncu, F., Uyanik, B. S., Onur, E., Yildirim, Y., et al. (2003a). Atherogenic profile in preeclampsia. *Arch Gynecol Obstet 268*, 45–47.

Var, A., Yildirim, Y., Onur, E., Kuscu, N. K., Uyanik, B. S., Goktalay, K., et al. (2003b). Endothelial dysfunction in preeclampsia. Increased homocysteine and decreased nitric oxide levels. *Gynecol Obstet Invest 56*, 221–224.

Villar, K., Say, L., Gülmezoglu, A. M., Merialdi, M., Lindheimer, M. D., Betran, A. P., et al. (2003). Eclampsia and preeclampsia: a health problem for 2000 years. In H. Critchley, A. B. MacLean, L. Poston, & J. J. Walter (Eds.), *Pre-eclampsia* (pp. 189–207). London: RCOG Press.

Vita, J. A., Keaney Jr., J. F., Raby, K. E., Morrow, J. D., Freedman, J. E., Lynch, S., et al. (1998). Low plasma ascorbic acid independently predicts the presence of an unstable coronary syndrome. *J Am Coll Cardiol 31*, 980–986.

Waktsuki, A., & Okatani, Y. (2000). Melatonin against the free radical-induced impairment of nitric oxide production in the human umbilical artery. *J Pineal Res 28*, 172–178.

Wallukat, G., Homuth, V., Fischer, T., Lindschau, C., Horstkamp, B., Jupner, A., et al. (1999). Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor. *J Clin Invest 103*, 945–952.

Walsh, S. W., & Wang, Y. (1993). Secretion of lipid peroxides by the human placenta. *Am J Obstet Gynecol 169*, 1462–1466.

Walsh, S. W., Vaughan, J. E., Wang, Y., & Roberts, L. J. (2000). Placental isoprostane is significantly increased in preeclampsia. *FASEB J 14*, 1289–1296.

Wang, Y., & Walsh, S. W. (2001). Increased superoxide generation is associated with decreased superoxide dismutase activity and mRNA expression in placental trophoblast cells in pre-eclampsia. *Placenta 22*, 206–212.

Wang, Y., Zhang, Y., Lewis, D. F., Gu, Y., Li, H., D. Granger, N., et al. (2003). Protease chymotrypsin mediates the endothelial expression of P- and E-selectin, but not ICAM and VCAM, induced by placental trophoblasts from pre-eclamptic pregnancies. *Placenta 24*, 851–861.

Wang, Y., Gu, Y., Zhang, Y., & Lewis, D. F. (2004). Evidence of endothelial dysfunction in preeclampsia: decreased endothelial nitric oxide synthase expression is associated with increased cell permeability in endothelial cells from preeclampsia. *Am J Obstet Gynecol 190*, 817–824.

Watson, A. L., Palmer, M. E., Jauniaux, E., & Burton, G. J. (1997). Variations in expression of copper/zinc superoxide dismutase in villous trophoblast of the human placenta with gestational age. *Placenta 18*, 295–299.

Watson, A. L., Skepper, J. N., Jauniaux, E., & Burton, G. J. (1998). Susceptibility of human placental syncytiotrophoblastic mitochondria to oxygen-mediated damage in relation to gestational age. *J Clin Endocrinol Metab 83*, 1697–1705.

Wiktor, H., Kankofer, M., Schmerold, I., Dadak, A., Lopucki, M., & Niedermuller, H. (2004). Oxidative DNA damage in placentas from normal and pre-eclamptic pregnancies. *Virchows Arch 445*, 74–78.

Williams, M. A., Woelk, G. B., King, I. B., Jenkins, L., & Mahomed, K. (2003). Plasma carotenoids, retinol, tocopherols, and lipoproteins in preeclamptic and normotensive pregnant Zimbabwean women. *Am J Hypertens 16*, 665–672.

Wisdom, S. J., Wilson, R., McKillop, J. H., & Walker, J. J. (1991). Antioxidant systems in normal pregnancy and in pregnancy-induced hypertension. *Am J Obstet Gynecol 165*(6 Pt 1), 1701–1704.

Woods, J. R., Plessinger, M. A., & Miller, R. K. (2001). Vitamins C and E: missing links in preventing preterm premature rupture of membranes? *Am J Obstet Gynecol 185*, 5–10.

Yura, T., Fukunaga, M., Khan, R., Nassar, G. N., Badr, K. F., & Montero, A. (1999). Free-radical-generated F2-isoprostane stimulates cell proliferation and endothelin-1 expression on endothelial cells. *Kidney Int 56*, 471–478.

Zaken, V., Kohen, R., & Ornoy, A. (2001). Vitamins C and E improve rat embryonic antioxidant defense mechanism in diabetic culture medium. *Teratology 64*, 33–44.

Zeteroğlu, S., Üstün, Y., & Üstün, Y. E. (2004). Placental and cord malondialdehyde and maternal and perinatal outcomes. *Int J Gynaecol Obstet 85*, 47–49.

Zhan, C. D., Sindhu, R. K., & Vaziri, N. D. (2004). Up-regulation of kidney NAD(P)H oxidase and calcineurin in SHR: reversal by lifelong antioxidant supplementation. *Kidney Int 65*, 219–227.

Zhang, C., Williams, M. A., Sanchez, S. E., King, I. B., Ware-Jauregui, S., Larrabure, G., et al. (2001). Plasma concentrations of carotenoids, retinol, and tocopherols in preeclamptic and normotensive pregnant women. *Am J Epidemiol 153*, 572–580.

Zhang, C., Williams, M. A., King, I. B., Dashow, E. E., Sorensen, T. K., Frederick, I. O., et al. (2002). Vitamin C and the risk of preeclampsia—results from dietary questionnaire and plasma assay. *Epidemiology 13*, 409–416.

Zou, M. H., Leist, M., & Ullrich, V. (1999). Selective nitration of prostacyclin synthase and defective vasorelaxation in atherosclerosic bovine coronary arteries. *Am J Pathol 54*, 1359–1365.

Zusterzeel, P. L., Rütten, H., Roelofs, H. M. J., Peters, W. H., & Steegers, E. A. (2001). Protein carbonyls in decidua and placenta of pre-eclamptic women as markers for oxidative stress. *Placenta 22*, 213–219.

Zusterzeel, P. L., Steegers-Theunissen, R. P., Harren, F. J., Stekkinger, E., Kateman, H., Timmerman, B. H., et al. (2002). Ethene and other biomarkers of oxidative stress in hypertensive disorders of pregnancy. *Hypertens Pregnancy 21*, 39–49.